

DWARFISM, SELF-INCOMPATIBILITY, AND FEMALE STERILITY
IN VACCINIUM ASHEI (READE)

By

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Abstract of Dissertation Presented to the Graduate School
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Dwarfism and female sterility found in 2 anomalous wild V. ashei plants from north Florida and self-incompatibility in selected V. ashei plants from north Florida and south Georgia were investigated.

A dwarf plant of V. ashei (W78-66) was found in a native population of V. ashei in Nassau County, Florida. The plant had the normal chromosome number for V. ashei ($2n=6x=72$), had normal meiosis and produced fertile gametes. Stomate size and density were normal; leaf area was smaller than for most V. ashei cultivars but was normal for V. ashei plants. W78-66 had shorter internodes than cultivars and native plants, which were due to fewer cells per internode. W78-66 responded to GA³ both in vitro and in vivo. W78-66 grew well in vitro, maintaining its relatively short stature and internode size.

Dwarf and normal-height plants were found in the progeny of W78-66 x tall V. ashei cultivars. Short-internode selections from this

progeny, when intercrossed, produced normal height, dwarf, and extreme dwarf plants; when crossed with tall cultivars, they produced dwarf and normal height plants; when backcrossed to W78-66, they produced normal-height, dwarf, and extreme dwarf plants.

Self-compatibility measured as fruit set, number of seeds per pollination, and number of seeds per fruit was highly variable among 19 native clones from north Florida and south Georgia. Self-fruitfulness in all clones was insufficient to allow block plantings of single clones in a production field.

The female-sterile V. ashei clone (W78-122) had extremely large leaves compared with other native and cultivated plants of V. ashei. Despite heavy flowering for 4 observed years, fruit set never occurred. Meiosis appeared normal, with $2n=6x=72$ chromosomes. Male fertility, measured as percent pollen germination on an artificial medium and fruit set of unrelated V. ashei cultivars pollinated with W78-122, was normal. V. ashei pollen germinated on the pistil and grew through the style of W78-122. Dissected ovaries from flowers at anthesis contained only a few healthy-looking ovules. Cleared ovules from flowers at anthesis indicated abnormal megagametophyte development. Female sterility ranged between 11 and 25% in 3 of 4 populations of fertile V. ashei cultivars x W78-122. All progeny of the fourth cross were female fertile.

SECTION I

INTRODUCTION

The beginning of the cultivation of blueberries dates only to the early part of this century. Prior to this time all blueberries were harvested from native stands. The successful development of the blueberry industry has been closely tied with Vaccinium breeding programs (Galletta, 1975). Dr. F.V. Coville of the United States Department of Agriculture directed the first such breeding program. He released the 3 cultivars -- 'Pioneer', 'Cabot', and 'Katherine' -- in 1920, and these served as the basis for the early blueberry industry (Coville, 1921).

All commercially-grown blueberries are derived from species within Vaccinium section Cyanococcus (Galletta, 1975). Cultivated blueberries are divided into 3 major groups: 1) native lowbush, 2) improved highbush, and 3) improved rabbiteye.

V. ashei, the rabbiteye, is the tallest of the cultivated blueberry species. As a native plant, it is adapted to the upland regions and stream beds of the southeastern United States. Major populations are found along the Satilla River in southeastern Georgia, the Suwannee River in northern Florida, and the Yellow River in the Florida Panhandle (Camp, 1945).

V. ashei is not restricted to these areas. In the 1920s, 1000 hectares of blueberry seedlings, mostly V. ashei, were collected from native stands and planted in Florida (Darrow, 1966). These blueberry plantations, now abandoned, contain an enormous amount of potentially valuable genetic variability (Lyrene and Sherman, 1979).

Utilization of this germplasm in a breeding program requires an understanding of the traits for which variability exists and information about their inheritance. It was the objective of this research to 1) investigate the nature and inheritance of dwarfism and female sterility, as found in 2 anomalous wild V. ashei plants from north Florida, 2) determine the nature and degree of self-incompatibility in selected V. ashei plants from north Florida and south Georgia.

SECTION II

LITERATURE REVIEW

Dwarfism in Plants

Mature plant height is the end result of many interacting biochemical and physiological pathways and is influenced by both genotype and environment. Environmentally-induced dwarf plants are common in areas such as mountain slopes, salt marshes, and dry regions. Plants reach their maximum height in areas most conducive to plant growth, such as the wet tropics, and are progressively shorter as the latitude or altitude increases. An example of man's exploitation of environmentally-induced dwarfing is Bonsai, in which pruning and shaping, combined with growing in small containers, maintain a normally large plant at a small size for periods up to century or more.

Genetic dwarfs are common throughout the plant kingdom. Nonvigorous dwarfs may arise due to semilethal genes or incompatibility within the genome or between the genome and cytoplasm. On the other hand, genes exist in the gene pools of many plant species that can produce vigorous but dwarf plants. There are basically 2 types of vigorous dwarfs. In one type, called semidwarfism, only the internodes are shorter than normal. Short-internode dwarfs can be extremely important in agriculture and horticulture. The second type of dwarfism could be called complete dwarfism, because it results in plants in which all organs are smaller than normal.

Genetically dwarf plants may be dwarf because of 1) fewer cells due to fewer cell divisions, 2) smaller cells due to less cell elongation or early secondary cell wall thickening, 3) a combination of 1 and 2. Bindloss (1942) reported that there were fewer cell divisions in the stem of a dwarf Lycopersicon esculentum L. compared with the normals. In the dwarf 4963 of Zea mays L., O'Donald reported fewer epidermal cells in the coleoptile than in normal plants (Pelton, 1964). The epidermal and coleoptile parenchyma cells averaged shorter in Dwarf-1 of Zea mays than in normals (Pelton, 1964). Shorter epidermal cells have also been reported for the root of 'wilty dwarf' of Lycopersicon esculentum (Lee, 1958) and for leaves of a dwarf Lolium perenne L. (Cooper, 1958).

A combination of fewer cell divisions and less cell elongation has been reported for various tissues of 'Dwarf-1' versus normal sibs in Zea mays. The combination was reported in the rib meristem which produced the internodal tissue (Abbe and Phinney, 1940), in the parenchyma (Hansen and Abbe, 1943 as cited by Pelton, 1964), in the cortex (Hansen, 1957), and in the axis of the mesocotyl and parenchyma of the first leaf sheath (Skjægstad, 1958 as cited by Pelton, 1964).

Precocious secondary cell wall thickening was found in the dwarf columbine (Aguilegea vulgares L. 'compacta') (Pelton, 1964). Early cell wall thickening inhibited cell elongation, resulting in a dwarf, compact growth habit. Brittle stems and erect flower buds were pleiotropic effects of the same gene.

Hormones have been shown to play a vital role in the control of growth of the whole plant as well as of individual organs. Three classes of growth-promoting hormones that have been investigated in

reference to dwarfs are auxins, cytokinins, and gibberellins (Thimann, 1977).

Auxin is required for cell elongation (Thimann, 1977). Internode tissues which are elongating most rapidly have the highest level of endogenous auxin (Wareing and Phillips, 1978). Treatment with indoleacetic acid did not increase the growth of dwarf Tephrosia vogelli Hook. (Irving and Freyre, 1960), Lycopersicon esculentum (Plummer and Tomes, 1958) and Ipomea nil L. (Pelton, 1964). Phinney (1956) reported that 7 single gene dwarfs of Zea mays did not respond to several different auxins or hydrolyzed casein and coconut milk. However, dwarfs may be either sensitive or insensitive to exogenous applications of plant hormones. Reduced sensitivity to exogenously applied auxin was reported in 'Dwarf-1' in Zea mays (Overbeek, 1938 as cited by Teas, 1957). High rates of auxin destruction in the plant were found to be the reason for short stature. Low auxin sensitivity in this mutant also explains its insensitivity to applied auxins.

Interaction of auxins and cytokinins was shown by Sachs and Thimann (1967) to control apical dominance. Applied cytokinins are known to stimulate growth of dormant axillary buds (Sachs and Thimann, 1964; Williams and Stahly, 1968). Shootiness, which is characteristic of many dwarf types, results because of the growth of many axillary buds, and may be due to high levels of endogenous auxin in the axillary buds (Wareing and Phillips, 1978). Auxin can direct the transport of cytokinins from the root where they are synthesized.

Optimum shoot number was obtained when 3 strains of 'McIntosh' apples (Malus spp.) were grown in vitro at a cytokinin level of between 3-6 μM 6-benzylamino purine (BA) (Lane and Looney, 1982). When 10 μM BA

was used, only the extremely compact strain 'McIntosh Wijcik' and the compact 'Macspur' continued to proliferate more new shoots than the standard varieties, and since they all had a similar cytokinin optimum in vitro, dwarfness is probably not due to differences in endogenous levels of cytokinin. The abilities of these compact apple clones to metabolize excess levels of cytokinin or to counter the high cytokinin levels with higher or lower levels of other hormones were given as possible reasons for the continued shoot proliferation at high cytokinin levels (Lane and Looney, 1982).

Gibberellins (GA) were first studied by Japanese botanists. A rice disease caused by the fungus Gibberell fujikuroi Idn. produces plants which are much taller and thinner than the uninfected plants. Yabuta and Sumiki (1938 as cited by Stowe and Yamake, 1959) isolated two crystalline, biologically active compounds which they named gibberellins A and B. Today 52 different GA structures are known. It is now clear that gibberellins are naturally-occurring compounds in higher plants. Gibberellins are found in all parts of higher plants, and the major GA in intact plants is GA₁.

Brian and Hemming (1955) reported that only a minute quantity of gibberellic acid (GA) was required to increase the growth rate of dwarf peas to that of a normal variety. Since that time numerous studies have been conducted on the effects of GA on plant growth. The activity of GA is not limited to height; it also increases leaf expansion, branch angle, parthenocarpy, bolting of long day plants, and sometimes eliminates vernalization requirements and removes light inhibition to seed germination (Weaver, 1972). But, the effect on dwarf plants is probably the most dramatic.

Hedden, MacMillan, and Phinney (1978) list 3 processes -- metabolic control, deactivation, and compartmentization -- which can regulate GA levels in higher plants. Light, temperature, and other hormones also are known to affect GA levels (Weaver, 1972).

Metabolic control involves enzymatic regulation at one of the branch points in the GA biosynthetic pathway. The GA pathway has a common intermediate with the phytol and carotenoid pathway. Hedden and Phinney (1967) correlated the absence of GA-like substances in the 'Dwarf-5' mutant of Zea mays with low rates of the GA intermediate ent-kaurene synthesis.

The 2B hydroxylation of GA's is probably the most widespread type of deactivation of gibberellic acid (Hedden, MacMillan, and Phinney, 1978). Rappaport et al. (1974) used radioactive GA's to establish direct evidence for 2B-hydroxylation by higher plants. The reaction is not known to be reversible and results in a marked reduction in biological activity.

Only recently has it been shown that the complete GA biosynthetic pathway operates in plastids (Hedden, MacMillan, and Phinney, 1978). The phytochrome red/far red light reaction has been shown to affect the level of extractable GA substances from plastids (Stoddart, 1976). Compartmentization of GA synthesis and phytochrome-mediated release may be an important method of GA regulation (Cooke and Kendrick, 1976).

While GA's have been shown to affect a number of growth processes, the effect on stem elongation has received the most attention. Brian and Hemming (1955) reported differences in height between treated and untreated dwarf peas (Pisium sativum L.) of 500% after 1 application of GA₃. The response was generally limited to younger tissues which were still growing and could be influenced by both genotype and environment.

Increased cell length has usually been reported when anatomical examinations were made after GA treatment. Kurosawa (1926 as cited by Stowe and Yamaki, 1959) showed that with rice (Oryza sativa L.) plants infected with G. fujikuroi that the epidermal and parenchymatous cells of leaves and internodes were longer and slightly reduced in radial and tangential diameters compared with cells in uninfected plants. Increased cell divisions have also been reported after treatment with GA's (Stowe and Yamake, 1959). Katsumi et al. (1964) stimulated seedling elongation in the 2 maize dwarfs, 'Dwarf-5' and 'Anther-ear-one, by treating with GA precursors, but 'Dwarf-1' and 'Dwarf-2' were not stimulated by the same treatments. Presumably, determination of the nature of a dwarf is possible by noting whether or not the dwarf plant elongates when treated with compounds representing different steps in the GA biosynthetic pathway.

Response to exogenous GA varies with dosage, environment, and frequency of application (Pelton, 1964). Phinney (1956) found that the degree of response was positively correlated with dosage in 4 dwarf mutants of Zea mays. Daily applications of very small dosages of GA₃ to 'Dwarf-1' and normal seedlings increased growth to the lethal point. All plants died, and the stem length of the dwarf was similar to treated normals (Pelton, 1964).

Insensitivity to exogenously-applied GA was shown for the 3 "major" genes for reduced height in Triticum aestivum L.: Rht 1, Rht 2, and Rht 3 (Radley, 1970). Radley reported that the tissues of some Norin-10 dwarfs contained increased levels of endogenous GA over some tall plants and concluded that dwarfness was due to a block in the utilization of GA, not a block in the production of GA.

Genetic dwarfism has been found in numerous plant families. Pelton (1964) listed 112 dwarf forms in 34 genera of 17 families of angiosperms. Den Ouden and Boon (1965) listed numerous genetic dwarfs in many species of conifers.

Vavilov (1951) used dwarfism to exemplify homologous variation in plants, and he predicted that dwarf forms could be found throughout the plant kingdom. Although dwarfism has been found and successfully used in many crop plants, the genetic pathways to dwarfism appear to be numerous and diverse, and uncertainty still exists over the inheritance of plant height. Polygenic, oligogenic, and monogenic control, both dominant and recessive, have been listed, often for the same crop. Interactions with other characteristics such as number and angle of branches, vigor and genetic background, have been postulated as possible reasons for unclear results from some inheritance studies. High heterozygosity, large plant size, a long juvenility period, and, in some cases, polyploidy, have limited genetic studies of dwarfism in woody perennials.

In hexaploid bread wheats (T. aestivum), polygenic control of height was indicated by early studies (Freeman, 1919; Clark, 1924; Torrie, 1936). Reduced height was brought about by new combinations of already-present genes (Gale and Law, 1977). Two independent major genes for dwarfing Rht-1 and Rht-2, were introduced through the Japanese Variety Norin-10 (Fisk and Qualset, 1973). Another dwarf gene, Rht-3, from variety 'Tom Thumb', was found to be allelic to Rht-1 (Fisk and Qualset, 1973). Allen, Vogel, and Peterson (1968), showed that the Norin-10 and Brevor-14 varieties carried 2 genes determining semidwarfism. Yet, in some varieties, such as Suvon-92, homozygosity

for only 1 of these 2 genes was required to produce the semidwarf phenotype. The 2 genes are additive in their effects and the alleles determining dwarfism at each locus are recessive (Fisk and Qualset, 1973).

Crosses between Norin-10 semidwarf wheat and conventional lines did not result in discrete F-2 classes as had been expected. The F-2 frequency distribution for plant height showed continuous variation. This was true whether or not populations were segregating for the major genes (Gale and Law, 1977). The removal of vernalization and photoperiodic effects by growing progeny of 8 segregating wheat lines in a greenhouse was necessary to reveal the expression of almost complete dominance of tall plants (Halloran, 1975).

Polygenic control of height as well as "major" qualitative genes exist in rice (Oryza sativa) (Foster and Rutger, 1978). A single recessive gene for dwarfness from the variety 'Dee-geo-woo-gen' was reported by Aquino and Jennings (1966). At least 3 nonallelic semidwarfing genes--sd1, sd2, and sd4--have been induced by irradiation in the rice cultivar 'Calrose' (Mackill and Rutger, 1979).

Legg and Collins (1982) reported that while the inheritance of short internodes from a short-internode tobacco (Nicotiana tabacum L.) was monogenic, expression of the trait was highly affected by year, maternal parent, cultivar background, and percentage of germplasm from the mutant parent. The variation in expression was only found when the gene was heterozygous. When homozygous, it was totally epistatic over any quantitative genes, and all homozygous recessive plants were fully dwarf.

Several types of dwarf plants have also been described in peaches, Prunus persica L. Batsch. Hesse (1975) listed 2 types of dwarf trees: Brachytic dwarfs (dw) and bushy (bu_1 and bu_2). Lammerts (1945) studied the inheritance of both dwarf types. All of the F-1 progeny of a cross between the brachytic dwarf and normal plants were normal in height. In the F-2 a clear 3:1 normal:dwarf segregation was obtained. The dwarf parent and progeny had very short internodes, and at the end of 2 years were approximately 1/5th the height of the normal progeny.

Analysis of tree types has been conducted with peach plants having varying levels of the dw gene in different genetic backgrounds (Scorza, 1984). Phenotypic variations resulted from interactions of internode and branch length, bud break, and branch angle. The homozygous recessive (dw_1dw_1) was commercially undesirable due to excessive dwarfism which resulted in plants being too short and the foliage too dense.

The bushy genes (bu_1, bu_2) in peach were discovered in a segregating population from "close pollination" of the commercial variety 'Babcock' (Lammerts, 1945). Of the 329 seedlings from the cross, 308 were normal tall plants and 21 were "somewhat brachytic, bushy types." These plants had shorter internodes and the branches were thicker than the tall types. Nonallelism of the 2 dwarf types was determined by intercrossing.

Reduction in tree size in apples has been correlated with short internodes (Brown, 1975; Lapins, 1976). Dwarfism was originally found as a spontaneous mutant in some apple cultivars and has also been induced by mutagens (Lapins, 1974, 1976). Zagaja and Faust (1983) correlated tree size and vigor. Continuous variation was found in tree

size due to vigor, number of branches, and internode length. They concluded that because of the observed continuous variation, the traits were under polygenic control. Decourte (1967 as cited by Brown, 1975) showed that dwarfness (non-spur type) is controlled by the single recessive gene n and a number of cultivars are Nn. Polygenic control has been reported for the spur type growth habit in 59 of 60 progenies of apple (Lapins, 1976). In this report, one progeny from the cross 'Golden Delicious' x 'McIntosh Wijeik' segregated 1:1 spur to non-spur type, which indicated that the spur type was segregating for a dominant gene conditioning the spur phenotype.

Dwarfism has also been reported in Prunus avium L. (Fogle, 1961), Pyrus spp. L. (Tukey, 1964), Rubus ideaus L. (Keep, 1969), and Citrus spp. L. (Cameron and Frost, 1968).

Dwarfism, like most traits in Vaccinium, has not been thoroughly investigated as to mode of inheritance. No dwarf genes in any of the Vaccinium species are reported in the literature. There are, however, definite differences in height among species. Height ranges from the low, less than 30 cm tall, rhizomatous, lowbush types to the very tall (2-5m) rabbiteye and highbush types (Eck and Childers, 1966). Natural selection for adaptation to different environments would tend to "fix" height within a population. Advantages of short stature include: winter survival under snow cover, increased drought tolerance, greater fire tolerance, and decreased vulnerability to wind. On the other hand, in the habitats populated by highbush blueberry species, greater stature allows the plants to compete more effectively for sunlight.

Crosses between highbush and lowbush species have been made by several investigators (Johnston, 1946; Meader, Smith and Yeager, 1954;

Darrow, Morrow and Scott, 1952). Johnston (1946) found that the lowbush growth habit was almost completely dominant in crosses between lowbush and highbush types. He reported that 97% of the progeny were lowbush types.

The inheritance of bush type in hybrids of highbush and lowbush blueberries was studied by Meader, Smith, and Yeager (1954) in New Hampshire. All F_1 plants were intermediate in height and definitely taller than the lowbush parents. The F_2 population after 13 years in the field was grouped into 3 classes: 1) lowbush (30 cm or less); 2) intermediate (31 cm to 1 m); and 3) highbush (1 m or more). Segregation for bush type of 954 seedlings was 246 lowbush, 699 intermediate, and 9 highbush. While dominance of the lowbush type is not readily apparent, neither the intermediate height of the F_1 nor the artificial grouping of the F_2 argue against the findings of Johnston.

Darrow, Morrow, and Scott (1952) reported on the characteristics of highbush x lowbush blueberries progenies grown in New Jersey, Maryland, and North Carolina. Most of the F_1 hybrid population ranged from 75 to 100 cm in height and were relatively uniform in height. The BC_1 plants (half-high backcrossed to highbush) had a height ranging from half-high (75 to 100 cm) to 2 m. Plants of a small F_2 population were grown and found to be vigorous and no higher than the half-high F_1 plants. At the time of measurement, plants were fairly uniform in height but were starting to show variability in spreading habit.

Dwarfism as expressed in the Vaccinium selection W78-66, may be a product of: interspecific hybridization between native highbush and lowbush species; an extreme segregate from a normal curve distribution in "height" genes; a rare allele that became homozygous; or a new

dominant mutation. It was the objective of this research to gain an understanding of: the origin of dwarfism as expressed in W78-66; the nature of the dwarf trait; and how the trait is inherited.

Self-incompatibility

In 1793, Cristian Sprengel wrote, "It seems that nature is unwilling that any flower should be fertilized by its own pollen" (translated by Lovell, 1918 p.12). Similar observations have subsequently been made by numerous authors as detailed by many reviewers of pollination biology (Baker, 1979; Frankel and Galun, 1977; Nettancourt, 1977; Lewis, 1949; and Schmid, 1975).

Mechanisms designed to prevent inbreeding can be divided into 2 groups based on their time of action-- prefertilization and postfertilization. Prefertilization barriers consist of dichogamy, dioecy, and self-incompatibility. Postfertilization barriers include death of the zygote, reduced viability, and possibly sterility of the resultant seedling.

Self-incompatibility has been defined in various ways (Brewbaker, 1957; Arasu, 1968; Lewis, 1949; Lundqvist, 1964). The definition of self-incompatibility in higher plants as "the inability of a fertile hermaphrodite seed-plant to produce zygotes after self-pollination" (Lundqvist, 1964 p.221) avoids several weaknesses of other definitions. Nonfunctional male and female gametes as well as other pre- and postfertilization barriers are distinguished from self-incompatibility.

The mechanism of the self-incompatibility system in flowering plants is the blockage or restriction of incompatible pollen growth to

the ovule. Self-incompatibility has been divided into sporophytic and gametophytic systems. In sporophytic systems pollen's compatibility phenotype is determined by the genotype of the pollen-producing parent. In gametophytic incompatibility systems the genotype of the individual microspore determines the recognition reaction. Time of gene action, site of expression, and presence or absence of floral polymorphism are often different the 2 incompatibility systems. The incompatibility reaction is expressed in the female part of the flower at the time of flower opening in both sporophytic and gametophytic systems (Nettancourt, 1977).

Sporopollenin deposited at the time of tapetal breakdown into the cavities of the pollen exine is responsible for the sporophytic control of self-incompatibility (Heslop-Harrison, 1975; Dickinson and Lewis, 1973).

The incompatibility reaction in the gametophytic system occurs during pollen tube growth in the style. In the gametophytic incompatibility system, abnormal behavior of the generative and vegetative nuclei of incompatible pollen tubes (Nettancourt, 1977) was reported. In incompatible pollen tubes, the generative nucleus did not divide, and the vegetative nucleus disappeared within the first few hours after germination. The vegetative nucleus was reported by Nettancourt (1977) to provide the genetic information for the incompatibility reaction in pollen tubes. Thickened pollen tube walls, an increase in the number of callose plugs, high respiration rate, increased acid phosphatase and cytochrome oxidase activity, and altered protein patterns have all been reported in incompatible pollen tubes (Nettancourt, 1977).

The stigma, style, and ovule have been listed as sites of the incompatibility reaction in different plants. Brewbaker (1959) discovered that the site of the reaction was closely associated with pollen cytology. The stigma was listed as the site of reaction of species with trinucleate pollen (pollen which has undergone the second mitotic division prior to anther dehiscence). This included grasses with homomorphic gametophytic incompatibility and many species with the sporophytic system. Incompatibility reactions within the style or ovary were reported for 36 genera of higher plants. Of these, 33 had binucleate pollen (pollen which has undergone only 1 mitosis at the time of dehiscence of the anther). The ovary as the site of the incompatibility reaction was, until recently, only reported in Theobroma cacao L. (Cope, 1962). It has since been reported to be the site of the reaction in Vaccinium (El-Agamy, 1979) and in several tropical trees (Bawa, 1979).

In sporophytic incompatibility systems, polymorphisms in flower morphology which reinforce outbreeding have developed in a number of species. Distyly and tristyly are 2 such systems. Species with distyly have 2 different flower types; those with tristyly have 3 flower types. Style and anther filament lengths differ in the flower, and pollinations of like flower types are incompatible. Differences in flower morphology are not the main barriers to self seed production in these plants, but are associated with the SI system (Darwin 1878). Differences in flower morphology are not associated with the gametophytic incompatibility system.

The genetic control of self-incompatibility in plants having the gametophytic system may be due to 1, 2, or even 3 loci, each having

several to many alleles (Nettancourt, 1977). The one, multiallelic locus (S) system was first reported in Nicotiana glauca Erd. (East and Mangelsdorf, 1925). Compatible pollination takes place only when a given S-allele in the pollen is met with different S-alleles in the pistil. The 2 multiallelic loci (S and Z) system was discovered by Lundqvist (1954) in rye (Secale cereale L.) and subsequently found in other species of the Gramineae. The two loci are inherited independently but are epistatic. Allelic identity at both loci in the genotypes of the pollen and the pistil leads to incompatibility, while identity at only 1 locus leads to a compatible pollination. At least 3 loci (S) have been estimated to be active in Ranunculus acris Hook. and possibly 4 in Beta vulgaris L. (Lundqvist, 1975).

The sporophytic self-incompatibility system is characterized as having a single locus (S) with several alleles responsible for genetic control (Nettancourt, 1977). Since the reaction of the pollen is determined by the genotype of the sporophytic tissue in which it was formed, the reaction in the pollen as well as the stigma is controlled by two S-alleles. The 2 S-alleles may react independently or they may interact by one being dominant over the other, and these relationships may exist in 1, both or neither the pollen or stigma. In addition, dominance and independence relationships of the S-alleles in the pollen and in the pistil may differ.

Sporophytic self-incompatibility is characteristic of the Cruciferae, Compositae, and Rubiaceae (Lewis, 1979). Gametophytic self-incompatibility is characteristic of the Rosaceae, Leguminosae, Onagraceae, Scrophulariaceae, Solanaceae, Ranunculaceae, and Chenopodiaceae (Lewis, 1979).

Permanent and temporary elimination of self-incompatibility are possible. Doubling the chromosome number, induction of self-fertility mutations, and transfer of compatibility alleles into an incompatible line can result in the permanent elimination of self-incompatibility (Frankel and Galun, 1977).

Tetraploids of Trifolium, Nicotiana, Prunus, and Petunia lost the self-incompatibility that was present in the diploids (Nettancourt, 1969). The production of self-compatible pollen by tetraploids in Oenothera organensis Hook. is limited to pollen grains that carry 2 different S-alleles (Lewis, 1947 and 1949). The compatibility of the S-heterozygous alleles was confirmed by Lewis and Modlibowska (1942). It is thought that competitive interaction between the different S-alleles leads to loss in expressivity for each of the 2 alleles involved.

Annerstedt and Lundqvist (1967) showed that polyploidization does not result in the loss of self-incompatibility in monocots. They suggested that genetic drift, selection, or an excess supply of S-metabolites in the pollen grain have enabled monocot self-incompatibility systems to tolerate gene duplication. Genetic background of the pollen grain, gene dosage, and stylar interactions are reasons given for the disturbance of the self-incompatibility reaction in triploids and aneuploids (Nettancourt, 1977).

Induction of self-compatibility using radiation breeding has had variable success. Both pollen and stylar mutations have been achieved (Nettancourt, 1977). The self-compatible allele in the sweet cherry 'Stella' (Prunus avium) resulted from irradiation of a self-incompatible cherry (Fogle, 1961).

Transfer of self-incompatibility by crossing a highly self-incompatible cultivar with a compatible cultivar has become an important part of hybrid seed production in some Brassica species (Odland and Noll, 1950). Ronald and Asher (1975) transferred compatibility from garden clones of Chrysanthemum x morifolium Ramat. into incompatible greenhouse cultivars. Compatibility appeared to be dominant.

Variation in degree of self-incompatibility has been reported within a number of plant species including some Vaccinium species (Meader and Darrow, 1947; Aalders and Hall, 1961; El Agamy, 1979). Variation in the activity or penetrance of the S-alleles and epistatic reactions with fertility genes are listed as possible reasons (Frankel and Galun, 1977). According to these authors, in most systems studied thoroughly, genes were found which interfered with strict incompatibility. The heritability and the type of genetic control of intraspecific variation in self-incompatibility seemed to be quite variable.

Pseudo-self-compatibility (PSC) is a temporary disruption in the self-incompatibility system. It can be found in the pollen and/or the pistil. Plants with PSC have a functional incompatibility system but produce seeds from self- or incompatible cross-pollinations in amounts ranging from less than 1% to 100% of a compatible cross. PSC is thought to be due to segregation of modifying genes. (Henny and Ascher, 1976; Litzow and Ascher, 1983).

Temporary bypassing of the incompatibility barrier has been achieved by irradiation of pollen mother cells, bud pollination, delayed pollination, heat treatment of the pistil, application of growth

regulators, maceration and surgical removal of the stigma, shortening of the style, double pollination (pollinating with mixed killed-compatible and live-incompatible pollen or pollinating with compatible pollen followed several hours later by pollinating with incompatible pollen). In addition, pollen has been injected directly into the ovary. In vitro techniques have also been used to overcome self-incompatibility. One technique has been to remove the ovules and place them in sterile culture with the incompatible pollen (Nettancourt, 1977).

Early pollination studies in Vaccinium revealed the presence of self-incompatibility. F.V. Coville (1921) stated that self-pollinated blueberry flowers yielded berries which were "fewer, smaller, and later in maturing" than cross-pollinated ones. Pollination experiments conducted over 3 years on 15 highbush varieties showed that cross-pollination usually increased the crop sufficiently to warrant interplanting of 2 or more varieties, even though fruit set was not always reduced by selfing (Meader and Darrow, 1947). Also, mature cross-pollinated berries contained a higher number of well-developed seeds and tended to be larger than the fewer-seeded, self-pollinated berries.

Flowers of 24 clones of the lowbush blueberry (V. angustifolium Ait.) were self-pollinated in the field during 1958 and 1959 (Aalders and Hall, 1961). Methods used to prevent cross-pollination were not reported. Fruit set was obtained on only 1 clone which had only 8 fruit. Of the 21 lowbush clones tested in the greenhouse, 12 were completely self-incompatible and 9 had varying degrees of fruit set. Aalders and Hall concluded that the same pattern of self-fertility reported in highbush blueberries (Morrow, 1943) existed in lowbush blueberries. There are moderately self-fertile clones, slightly

self-fertile clones, and self-sterile clones. The average levels of self-fertility in lowbush and highbush blueberry appeared to be very similar.

The 10 rabbiteye varieties (Vaccinium ashei) tested in 1943 (Meador and Darrow, 1944) were placed into 3 groups based on their degree of fruit set after hand selfing in the greenhouse. Seven were considered relatively self-infertile and had a fruit set between 0 and 11.6%. Two varieties set approximately one-third of the flowers self-pollinated compared to about 70% when crossed. The variety 'Blueboy' set fruit equally well when selfed or crossed, averaging about 75% set. In general, days to maturity increased and berry size and seed content were reduced with selfing versus cross-pollination. Similar findings were made with Florida highbush and rabbiteye varieties (El-Agamy et al., 1981; Hellman and Moore, 1983).

To determine the site of the incompatibility reaction in highbush and rabbiteye clones, pollen tube growth in the pistil was monitored by El-Agamy (1979). He found that self-pollination of highbush and rabbiteye clones gave no significant difference in pollen germination and growth through the style. There were no visual differences in callose deposition on pollen tube walls in compatible and incompatible matings. Also, pollen tubes entered the ovary cavity in both pollination types, and in several cases, pollen tube growth into an ovule occurred in normally incompatible matings. The rate of growth of pollen tubes in the style was shown to vary with type of cross (incompatible vs. compatible) and temperature in which the plant was growing (Knight and Scott, 1964). Incompatible pollen tubes grew much slower than compatible pollen tubes at both temperatures tested (high,

16-27° C and low, 8-24° C). At the high temperatures incompatible tubes grew much faster than at lower temperature.

Despite the incompatibility barrier to self-pollination in Vaccinium, new cultivar development has relied upon a very narrow genetic base. Hancock and Siefker (1982) reported that coefficients of inbreeding (F) ranged from 0.00 to 0.25 for the 63 highbush blueberry cultivars released by public agencies in the United States. Three native selections ('Brooks', 'Sooy', and 'Rubel') contributed most of the genes in present-day cultivars. The inbreeding coefficients of most rabbiteye blueberry cultivars is also considerable, and most of the cultivated germplasm can be traced to the 4 landraces, 'Ethel', 'Myers', 'Clara', and 'Black Giant' (Lyrene, 1983).

Plant vigor (height, shoot diameter, and survival) was greatly reduced after one generation of selfing of 2 cultivars and 6 native selections of V. ashei compared with outcrossed populations (Lyrene, 1983). Two highbush cultivars demonstrated a significant negative correlation between mean seedling fresh weight and level of inbreeding (Hellman and Moore, 1983).

The high degree of self-incompatibility in most present cultivars of blueberries may be due to chance selection of high self-incompatibility in the foundation clones, or alternatively, it may reflect a generally high level of self incompatibility in V. ashei. The objectives of this research were to measure the degree of self-incompatibility in V. ashei selections from native populations in north Florida and south Georgia.

Female Sterility in Plants

Sterile plants lack functional male and/or female gametes. The sterility may be due to chromosome aberration (inversions, translocations, deletions), aneuploidy ($2n+1$, $2n-1$, etc.), odd-ploidy ($1x$, $3x$, $5x$, etc.), chromosome incompatibility after wide-hybridization, or genic factors (nuclear or cytoplasmic). Incomplete chromosome pairing during meiotic prophase can result in uneven distribution of chromosomes and the loss of genetic information in the gametes. These gametes are often nonfunctional. Chromosome aberrations, aneuploidy, odd-ploidy, and wide-hybridization can decrease chromosome pairing, and fertility is often reduced.

Genic factors causing male and female sterility have been reported for a number of crops. Mutants were found in Zea mays which suppress the pistillate inflorescence (sk, ba) or convert the normally staminate tassel to pistillate (ts). All 3 genes are expressed only when homozygous (Frankel and Galun, 1977). In Rubus idaeus (Lewis and John, 1968), a normally hermaphroditic plant, genes suppressing stamen differentiation (m) and carpel differentiation (f) were found. Both genes are recessive and act independently.

Genic sterility factors have also been reported in bananas (Musa spp. L.) (Simmonds, 1953), citrus (Citrus spp. L.) (Frost and Soost, 1968; Osawa, 1912 cited by Jackson, 1972), peppers (Capsicum spp. L.) (Wilson et al. 1982; Kormos, 1954; Bergh and Lippert, 1965), alfalfa (Medicago spp. L.) (Bingham and Hawkins-Pfeiffer, 1984), peas (Pisium sativum L.) (Blixt, 1978), and tomatoes (Lycopersicon esculentum) (Rick and Butler, 1956). Male sterility in plants is reported more often than female sterility.

Female sterility may be due to lack of viable female gametophytes or other necessary structures. Breakdown of the integument and the nucellus and subsequent starvation of the fertilized embryo sac is associated with female sterility in Vaccinium angustifolium (Hall et al., 1966). Slow development of the integument was observed in 1 type of female sterile alfalfa (Bingham and Hawkins-Pfeiffer, 1984). The inner integument was absent and the outer integument did not completely close over the nucellus. As a result, both the nucellus and the female gametophyte were ruptured and destroyed. Childers (1960) reported that in another female sterile alfalfa, 85% of the ovules lacked a female gametophyte and the remainder had abnormal gametophytes. In Capsicum annuum L., 6 types of female sterility have been reported (Wilson et al., 1982). Two were due to abnormal ovules, 2 had a deformed style, 1 lacked integuments, and in 1 the cause of sterility was not reported. In most cases of genic female sterility, male fertility was reported as normal.

Megasporogenesis and megagametophyte development in Vaccinium have been studied by several researchers (Bell, 1957; Stushnoff and Palser, 1970; Edwards, 1970). Ovules are barely visible and appear as small white mounds on the placentae during the dormant season previous to flowering (Stushnoff and Palser, 1970). The megaspore mother cell develops directly from the micropylar archesporial cell. There is no parietal tissue and the megaspore mother cell is surrounded only by the nucellar epidermis. Regular meiotic divisions of the megaspore mother cell usually result in a linear tetrad, with the 3 micropylar megaspores disintegrating, leaving the chalazal megaspore to function. This cell divides 3 times to produce an 8 nucleated mature embryo sac which

corresponds to the Polygonum type (Maheshwari, 1950 cited by Stushnoff and Palser, 1969).

Ovular tissue continues to develop concomitant with megasporogenesis and megagametogenesis. The tissues surrounding the ovule continue to bend until at the time of meiosis the ovule is completely anatropous. A single integument elongates, closing over the tip of the nucellus to form the micropyle. The nucellus is completely broken down by the late 4-nucleate stage of the megagametophyte (Stushnoff and Palser, 1970). Megagametogenesis was reported to be completed at anthesis in 3 tetraploid advanced selections (Fla 4-15, Fla 4-71, and Fla 6-164) from the the Florida Vaccinium breeding program (Edwards, 1970).

The use of female sterility to increase hybrid seed production has been proposed in alfalfa (Childers, 1960), and peppers (Bergh and Lippert, 1965). Flower production declines in many plants as fruit set and develop, while in the "styleless" mutant of pepper, new flowers are produced for a longer period of time, providing a more continuous source of pollen for cross-pollination (Bergh and Lippert, 1965). Mixed planting of male and female sterile plants would allow harvest of all fruit without danger of getting selfed seed.

In all reported cases of female sterility in pepper, the malfunction has resulted from single recessive genes that are pleiotropic (Bergh and Lippert, 1965). Female sterility in alfalfa was due to a single recessive trait, tetrasomically inherited (Bingham and Hawkins-Pfeiffer, 1984). Hall et al. (1966) reported that multiple genetic factors may determine female sterility in Vaccinium angustifolium.

Male and female sterility have been reported in lowbush blueberries (Hall et al., 1966; Hall and Aalders, 1961). It was initially hypothesized that female sterility as expressed in the Vaccinium selection W78-122 could have been due to: chromosome aberrations, aneuploidy, odd-ploidy, chromosome incompatibility after wide hybridization, or genic factors affecting floral and fruit development. It was the objective of this research to determine the nature of the female sterility trait in W78-122 and its inheritance.

SECTION III
EXPRESSION AND INHERITANCE OF DWARFISM
IN VACCINIUM ASHEI (READE)

Introduction

The rabbiteye blueberry (Vaccinium ashei) is the tallest of the commercially-grown Vaccinium species. Three to 6 meters is the normal height for this species, but plants 9 meters tall have been reported (Eck and Childers, 1966). Differences in average height exist among the widely scattered populations of rabbiteye blueberries. West Florida populations are normally tall (about 5 to 6 meters), while south Georgia populations average 3 to 4 meters in height (Eck and Childers, 1966).

Natural selection for adaptation to different environments would tend to "fix" height within a population. Advantages of short stature include winter survival under snow cover for northern Vaccinium species, increased drought tolerance, greater fire tolerance, and decreased exposure to wind. The major disadvantage would be the inability of the short plant to compete with larger neighboring plants for sunlight.

Long intervals between breeding cycles and large plant size are 2 limitations to efficient and rapid improvement in many perennial woody plants. Dwarfism has been proposed as a way of reducing these problems (Hanche and Beres, 1980). Dwarf plants are often precocious, with shorter germination-to-flowering intervals. The use of dwarfing genes could allow more rapid progress in a breeding program by making possible more generations in a set amount of time. With dwarfs, more plants can

be grown per unit area. This allows the breeder to screen more plants, which increases the probability that superior plants will be found.

The benefits of dwarfism are not limited to the breeder; they also extend to the grower. Shorter intervals between planting and the first harvest mean a quicker return on initial investment. Estimated economic returns from standard size and genetically-dwarfed peach trees after 7 years' growth in California were compared by Hanche and Beres (1980). They reported that even though the initial investment was greater for the dwarf trees, the investment could be recovered 2-3 years earlier with dwarf trees than with standard size trees.

The objectives of the research described in this section were to 1) describe a short-statured, compact-growing V. ashei clone (W78-66) in its native environment and at the Horticultural Research Unit (HRU) near Gainesville, Fl., 2) determine the nature of its dwarfism, and 3) study the inheritance of this trait.

Expression of dwarfism in V. ashei W78-66.

Materials and Methods

Phenotype in its native environment

The clone W78-66 was found by Dr. Paul Lyrene on June 27, 1978. The plant was located at the edge of a predominantly pine forest facing SR 121, 11.8 kilometers north of Highway 90, northwest of Baldwin, Florida (Nassau County). Softwood cuttings and seeds were collected from the plant in July, 1978. Cuttings were rooted under mist and planted into the Vaccinium Germplasm Block (VGB) at the Horticulture Research Unit (HRU) near Gainesville, Florida. The open-pollinated seeds

were germinated the following winter, and seedlings were planted into a high density nursery at the HRU during the spring of 1979.

On March 6, 1985, W78-66 was studied in its natural habitat with 2 objectives in mind: 1) to describe the phenotype of the original dwarf; and 2) to see if the dwarf was qualitatively different from other V. ashei plants in the population or was merely an extreme variant from a normal-curve distribution. An abandoned blueberry plantation located near the town of Glen St. Mary's, Florida, was also studied. This plantation was probably planted between 1920 and 1940 using V. ashei from the western Florida panhandle (Lyrene, 1979), but many of the plants at the site were seedling progeny of the original plants. This site was selected for comparison of plant types. Measurements collected were 1) plant height, 2) colony diameter measured at 1 meter height, 3) number of stalks per colony, 4) trunk diameter, and 5) type of colony base.

Phenotype at the HRU

Two ramets of W78-66 were planted 20 cm apart in the VGB at the HRU near Gainesville, Florida in 1979. They have since grown together and were treated as one plant. These ramets were compared with wild V. ashei clones which were also planted in the VGB in 1979 and with 8 cultivars of V. ashei growing close by. The VGB includes clones of wild V. ashei from throughout north Florida and southeast Georgia. These clones were selected because of their good horticultural characteristics. Internode measurements were collected for W78-66, for 13 other members of the VGB, and for 8 cultivars of V. ashei growing in close proximity.

Internode lengths were determined for each plant by dividing the measured length of a stem by the number of internodes making up the stem. Ten stems were measured; all measurements were made on the third growth flush from the apex. This flush of growth was usually a light brownish-red color, signifying a moderate amount of lignification.

Chromosome count

Chromosome counts were made from mitotic cells of the apical meristem. Shoots from forced vegetative buds were collected when less than 1 cm long, fixed in 3:1 absolute ethanol:glacial acetic acid and held in the freezer until used (at least 24 hr). The outer leaves of the shoot tip were removed to expose the apical meristem. The apical meristem and a small piece of stem tissue were placed in 25% HCL for 20 min, and transferred to a pectinase-cellulysin solution [.03g pectinase plus .03g cellulysin (Calbiochem, San Deigo, CA) in 2 ml distilled water] for about 15 min. The tissue was squashed in 1% acetocarmine, destained in 45% acetic acid, and observed under phase contrast at 1000X.

Stomate size and density

Stomate size and number per unit area were measured on W78-66, 3 rabbiteye cultivars, 2 F-1 plants from the cross W78-66 x 6x V. ashei cultivar composite, and an extreme dwarf V. ashei (81-44) from the HRU. This extreme dwarf was a seedling from a V. ashei cultivar polycross in the rabbiteye breeding program. Leaf imprints were taken by applying fingernail polish to the underside of a newly mature leaf from each plant. The imprints were peeled off the leaves, placed on microscope

slides, and viewed under 250X magnification. Stomate length was measured using an ocular micrometer. Thirty stomates were measured for each leaf imprint. Stomate density was measured by counting the number of stomates in 10 different fields of view at 250X magnification.

Leaf area

Leaf size was measured on W78-66, 6 other native V. ashei clones, and on 4 rabbiteye cultivars to see how W78-66 differed from the other clones. Ten newly mature leaves were randomly taken to represent all sides of each plant. Two ramets of each cultivar and 1 ramet from each clone in the VGB were used with the exception of W78-66 where 2 plants were used. All plants were grown in an open field under full sunlight. Leaf size was measured using a LiCor Portable Area Meter Model LI 3000.

Cell length and number

Internode measurements were collected for W78-66, for an extreme dwarf F_2 segregate from the cross W78-66 x V. ashei cultivar, for a plant of normal height from the same cross, for another native V. ashei plant, and for 2 cultivars of V. ashei. Stem segments from the second oldest growth flush were collected. Internode measurements were made, a 1 cm segment of the internode was dissected using a Lancer Model 1300 Vibrotome, and longitudinal sections were placed on a microscope slide in glycerine. Cell number per segment and cell size were determined for each plant by counting the number of parenchyma cells in a column of cells and computing the average diameter. Cell size and number of cells per internode were subsequently calculated for each plant.

Gibberellic acid in vivo

The objective of this experiment was to determine whether the dwarfism expressed by W78-66 is due to the absence or to low levels of endogenous GA. Softwood cuttings of W78-66 and the V. ashei cultivar 'Tifblue' were rooted under mist during the fall of 1983. Rooted cuttings were potted into 15 cm pots January, 1984. Soil mix was 1:1 Canadian peat moss:sand v/v. On March 21, 1984, 12 plants of each clone were placed in full sun. All treatments were initiated at this time. GA at 0, 100, 250, and 500 mg/liter in deionized water and 4 drops of the surfactant "Tween" was applied to 3 plants of each clone. A pressurized sprayer was used and all solutions were applied to the point of runoff. A second application of GA_3 at the same levels was applied to the same plants in the same manner on June 21, 1984.

On August 15, 1984 plants were pruned, fertilized with Osmocote 18-6-12 at 42g per 15 cm pot, and placed in a greenhouse. The photoperiod was artificially lengthened using 1:1 fluorescent: incandescent lights to provide approximately 100 lux at plant level. Lights were set to provide a 4-hr night break between 10:00 PM and 2:00 AM. Plants were sprayed 3 times with GA_3 at 2-week intervals. GA_3 levels were 0, 500, 1000, and 2000 mg/liter. There were 3 plants per treatment. Plants remained in the greenhouse until December 17, 1984 when internode length of new growth on all plants was measured. Length of the new growth and number of nodes per new shoot were collected for W78-66.

Results and Discussion

Phenotype in its native habitat

The selection W78-66 was found not to be unique. Many V. ashei plants were observed and 13 plants were measured at the Baldwin site and 13 more at the Glen St Mary's site (Table 3-1). Three plants at the Baldwin site were found to have a very similar phenotype to W78-66. The dwarf phenotype was characterized by the combination of several traits. It was usually less than 2m tall and was extremely colonial, having many small-diameter shoots arising perpendicular to the ground. Each of the 4 dwarf types had a colony diameter of about 6m, with W78-66 having a colony diameter of 8m. The nondwarf types were usually 3m tall or taller. The canopy diameter was 1-2 meters; the colonies had less than 20 shoots with the largest 3 being greater than 2cm in diameter. In addition, most shoots arose from a narrow crown and angled outward.

While both the dwarf and tall colony types were found at both sites, there were differences in colony characteristics. The nondwarf types were much taller at the Glen St. Mary's site than at the Baldwin site. The dwarf colony types at the Glen St. Mary's site were more open and did not form the dense colonies that were found at the Baldwin site.

While it was helpful to divide the clones into dwarf and nondwarf types, this division was arbitrary. Intermediate types were found at both sites, and they showed variable expression in all the traits discussed.

Phenotype at the HRU

Compared to the standard V. ashei phenotype, W78-66 is a short-internode dwarf with a distinctive growth habit (Figure 3-1). It

Table 3-1. Characteristics of 27 *V. ashei* clones from 2 populations in northeast Florida.

Height of tallest stalks (m)	Colony diameter (m)	Shoots ^z (no.)	Diameter of largest stalks (cm)	Base N=narrow ^y W=wide
Glen Saint Mary's Site ^x				
3	1	8	>2	N
2	1	8	>2	N
4	1	4	>2	N
3	3	17	>2	N
1	3	>150	<2	W Dwarf type
1.5	3	37	<2	W Dwarf type
2.5	3	>150	<2	W Dwarf type
2	1	10	<2	N
2	1	8	<2	N
3	3	9	>2	N
3	2	8	>2	N
2	1.5	11	>2	N
2	2	18	>2	N
3	2	28	>2	N
W78-66 Site				
2	6	123	<2	W Dwarf type
3	2	27	>2	N
2	2	17	<2	N
2	2	6	>2	N
3	3	24	>2	N
3	6	75	<2	W Dwarf type
2	6	>50	<2	N
1.5	4	11	<2	N
3	6	25	>2	W
2	8	>150	<2	W W78-66
3	6	60	>2	N
2	6	>150	<2	W Dwarf type
2	4	>150	>2	W

^zNumber of shoots arising from either the base of the plant or from the roots.

^yShoots arising from a very narrow crown (N) or arising perpendicularly from the ground up to 1.3 m from the center of the colony (W).

^xPlants were descendants of a plantation made at Glen St. Mary's Nursery, probably before 1940. Original plants are believed to be from native populations from the western Florida panhandle.

Figure 3-1. Phenotype of W78-66 and a normal height V. ashei plant growing at the Horticulture Research Unit, Gainesville, FL. 1-a. W78-66 (dwarf). 1-b. W78-40 (normal height). 1-c. W78-66, typical shoot. 1-d. W78-40, typical shoot.



is very vigorous and highly fertile, both when open pollinated and when hand-crossed in the greenhouse with V. ashei cultivars. It has dark green foliage and rather small leaves which are semievergreen. The fruit are small and black, the plant very compact and spreading. As with other V. ashei, the terminal 4-6 nodes of the growing shoots die back at the end of each of several growth flushes the plant undergoes each year. In W78-66, shoots are concentrated in the upper third of the stem. Of these shoots, 2-3 are relatively large in diameter. The shoots form wide angles with the main stem. In addition, the shoots are of unequal length; the most apical shoots are shortest and those arising more basally get progressively longer. Consequently, all shoots from this main stem extend to approximately the same height. The main stem tends to grow outward and the axillary shoots grow straight which gives the plant a "layered" appearance.

The growth habits of all the cultivars and most of the other members of the VGB are quite different from the dwarf. The main stems tend to be upright. Lateral shoots arise from about the 4th node from the terminal and are evenly located the entire length of the stem. Most shoots are about the same length and have a small diameter. This gives the plants an upright, open appearance. Within the VGB there are intermediate types having one or several characteristics of W78-66 yet having longer internodes and one or more non-dwarf traits.

A possible reason for the differences in phenotype between W78-66 in its native habitat and at the HRU is environmental. In its native environment the plant was growing in the understory of tall pines, while the HRU plants are in full sun. The dwarf, in its native habitat, appears to be about 15 years old from seed. The ramets at the HRU were

started as rooted cuttings and are only about 6 years old. In both places, the dwarf appears different from most other V. ashei plants growing nearby. The dwarf at the HRU may take on the phenotype of the original plant as it gets older.

Internode length was shortest for W78-66 compared with both the cultivars and native V. ashei (Table 3-2). Cultivars tended to have longer internodes than native plants, but there were exceptions.

Chromosome count

Approximately 72 chromosomes were counted in each of 25 somatic cells of W78-66 undergoing mitosis, indicating that W78-66 is probably hexaploid with $2n=6x=72$.

Stomate size and density

Stomate size and density varied significantly among plants, but both W78-66 and the extreme dwarf 81-44 were within the normal range of V. ashei (Table 3-3).

Leaf area

The average surface area per leaf was significantly smaller for W78-66 than for all of the other V. ashei cultivars measured (Table 3-4). The dwarf also had the smallest average leaf surface area of the 7 wild V. ashei clones measured in the VGB, although it was not significantly smaller in leaf area than 2 of the wild clones. Area per leaf was quite variable, though cultivars usually had larger leaf areas than unimproved clones. There were exceptions, especially with W78-122, which was originally selected from the woods because of its large leaves and associated female sterility.

Table 3-2. Internode lengths of 13 cultivars and 8 native V. ashei clones growing at the HRU in Gainesville.

Clone	Type	Internode length (cm)	SD	Minimum length ^z (cm)	Maximum length ^z (cm)
Aliceblue	Cultivar	1.3a ^y	.2	1.1	1.7
Bluegem	"	1.3a	.2	.9	1.6
Beckyblue	"	1.3a	.2	1.0	1.6
Tifblue	"	1.2ab	.2	1.0	1.6
Bonita	"	1.1b	.1	.9	1.3
Climax	"	1.1b	.1	.9	1.3
Chaucer	"	1.1b	.1	.9	1.2
Delite	"	1.1b	.1	.9	1.3
W78-66	Wild	.6e	.1	.5	.8
W78-41B	"	1.3a	.2	1.1	1.9
W78-39	"	1.2ab	.3	.9	1.9
W78-122	"	1.2ab	.2	.9	1.4
Beulah 4	"	1.1b	.2	.8	1.2
W78-37	"	1.1b	.1	.8	1.3
W78-68	"	1.0bc	.2	.8	1.3
W78-75	"	1.0bc	.2	.8	1.3
W78-70	"	.9cd	.1	.8	1.1
Beulah 2	"	.9cd	.1	.8	1.0
W78-69	"	.9cd	.1	.6	1.1
Beulah 1	"	.9cd	.1	.7	1.0
W78-64	"	.8d	.1	.7	1.1

^zLowest or highest of 10 stems sampled per clone.

^yMean separation by Duncan's Multiple Range Test, 5% level.

Table 3-3. Stomate size and density in *V. ashei* grown in full sun at the Horticulture Research Unit, Gainesville, Fl.

Plant	Type	Stomatal pore length (μ)	Stomata per mm ²
Bluebelle	tall cultivar	45 a ^z	260 ab
Aliceblue	tall cultivar	37 b	274 a
Bonita	tall cultivar	39 b	270 a
W78-66	dwarf	38 b	250 b
82-165	(tall cv x dwarf, F ₁)	41 ab	241 b
82-194	(tall cv x dwarf, F ₁)	39 b	253 b
81-44	extreme dwarf	38 b	254 b

^zMean separation within columns by Duncan's Multiple Range Test, 5% level.

Table 3-4. Average area per leaf of natives and cultivars of *V. ashei*.

Plant	Type	Plant (no.)	Mean (cm ²)	SD	Minimum (cm ²)	Maximum (cm ²)
Bluebelle	Cultivar	20	10.7 b ^z	1.7	7.8	13.9
Climax	"	20	10.6 b	3.0	6.6	16.3
Fla K	"	20	16.9 a	2.2	13.5	20.9
Beckyblue	"	20	12.0 b	3.0	9.1	18.1
W78-66	Wild	10	5.3 c	1.4	3.0	6.5
W78-122	Wild	10	25.6 a ^z	5.3	19.2	36.9
W78-35	"	10	11.8 b	2.6	8.3	15.8
W78-39	"	10	8.4 c	1.4	5.1	10.3
W78-74	"	10	8.2 c	1.4	5.6	10.2
W78-37	"	10	6.8 cd	1.2	4.4	8.2
W79-34	"	10	6.0 cd	.8	4.0	6.7
W78-66	"	10	5.3 d	1.4	3.0	6.9

^zMean separation within columns within groups by Duncan's Multiple Range Test, 5% level.

Small leaf size in W78-66 may be due to a pleiotropic effect or close linkage with the gene or genes responsible for the short stature. But, since small leaf size is not unique to the dwarf and is found in several other native V. ashei, a more interesting question may be why the cultivars have such large leaves.

Cell length and number

In general, short-internode plants had smaller pith parenchyma cells than long-internode plants (Table 3-5 and Figure 3-2). The 2 dwarf types (W78-66, 84-172) had the smallest cells and the shortest internodes. While there were minor differences in cell size between the dwarf and nondwarf types, differences between the 2 types in cell number per internode were very large, indicating that the short-internode trait in W78-66 is due predominantly to fewer cells per internode compared with nondwarf plants.

A seedling from a cross between 2 wild V. ashei clones (84-171), was growing in the same test plot as the extreme dwarf F-2 segregate (84-172) from the cross W78-66 x tall cultivar, and was included as a check plant to compare with 84-172. This plant was selected as a representative of nondwarf types. Cell size and internode length of this nondwarf were approximately twice the size of the extremedwarf, while number of cells per internode was about the same for the 2 plants. This indicates that differences in internode length are due to differences in cell size more than cell number in this situation.

Table 3-5. Internode length, cell length, and cell number per internode in dwarf versus nondwarf V. ashei.

Plant	Type	Internode length (cm)	Cell diameter (μ)	Cells per internode (no.)
W78-66	dwarf	.7	22	290
84-172	dwarf selection	.5	18	260
Tifblue	tall cultivar	1.5	27	530
Bluebelle	tall cultivar	1.5	29	520
W78-122	tall selection	1.5	29	520
84-171	tall selection	.9	33	280

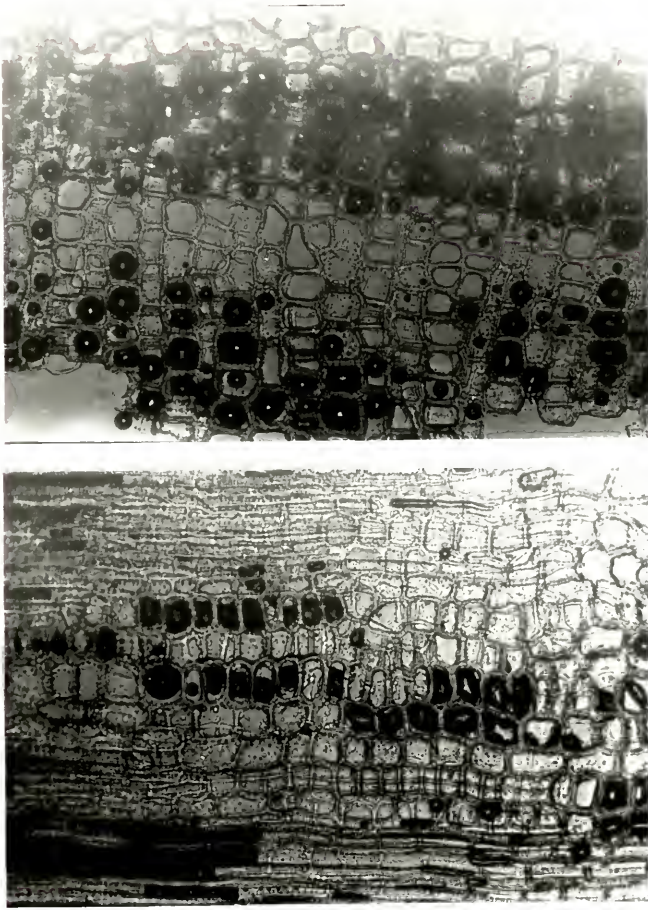


Figure 3-2. Pith parenchyma cells in internodal regions from newly mature stem segments from V. ashei W78-66 (top) and 'Tifblue' (bottom).

Gibberellic acid in vivo

Differences in phenotype were observed between the dwarf and nondwarf clones and within treatment levels of the dwarf. The new shoots of both clones had light green leaves. The oldest leaves of the new shoots were normal size and color, but the newest leaves were unusually small and chlorotic. Tip necrosis was present on most new shoots of plants which were treated with GA_3 . A high degree of necrosis of the new growth of the nondwarf cultivar 'Tifblue' did not allow length of new shoots or number of nodes of new shoots to be recorded.

Internode length was determined as the mean internode length of 4 continuous internode regions in the mid-portion of the new stem. This measurement was collected for both clones (Table 3-6). Internode length of both clones was increased by exogenously applied GA_3 . Differences between genotypes were significant. The "Dwarf" fit a cubic equation only slightly better than a linear regression indicating that the effect of GA_3 conc. on internode length was approximately linear in the range tested.

Length of new shoots, number of nodes per shoot, and internode lengths were measured on W78-66. All parameters mentioned increased with the increasing levels of GA_3 (Table 3-7).

Stem and internode elongation in response to GA_3 application has been studied by many researchers (see Pelton, 1964). Chlorotic leaves have also been reported after GA_3 treatment. This is believed to be due to failure of chlorophyll synthesis to keep pace with increased cell expansion--not to gibberellin interference with chlorophyll metabolism (Pelton, 1964). Plant necrosis was reported (Pelton, 1962 cited by Pelton, 1964) after daily applications of GA_3 . Tip necrosis of the treated 'Tifblue' plants in the present study may be a similar response.

Table 3-6. Internode length of dwarf and nondwarf types as affected by exogenously applied GA₃.

Clone	GA ₃ level	Stem segments (no.)	Internode length (cm)	
			Mean	SD
W78-66				
	0 mg/liter	30	1.1	.81
	500 "	24	1.6	.50
	1000 "	10	1.3	.88
	2000 "	10	1.7	.71
Tifblue				
	0 mg/liter	20	1.3	.43
	500 "	19	1.4	1.10
	1000 "	20	1.6	.87
	2000 "	19	1.9	1.40
Significance ^z				
Genotype				
Linear			**	
GA ₃ level				
Linear			**	
Quadratic			ns	
Cubic			ns	
Interaction				
Linear			*	

^z** , * Significant at .01 and .05 level respectively, and ns=not significant.

Table 3-7. New growth of W78-66 as affected by level of GA_3 .

GA_3 level	Length of new shoots (cm)	Nodes (no.)	Internode length ^z (cm)
0 mg/liter	8.1	11.0	1.09
500 "	12.7	15.0	1.13
1000 "	15.6	18.0	1.21
2000 "	20.5	21.0	1.50
Significance ^y			
Linear	**	**	**
Quadratic	ns	ns	ns
Cubic	ns	ns	ns

^zMean of 4 internodes located in the middle of the new shoot, not the total length of the new shoots/number of nodes.

^y**, * significant at .01 and .05 level respectively, ns=not significant.

The fact that internode length of the dwarf increased to levels found in the nondwarf after treatment with GA_3 suggests that W78-66 is dwarf due either to insufficient levels of active GA being produced by the plant or high levels of active GA deactivation.

Growth of *Vaccinium ashei* W78-66 in vitro.

Materials and Methods for Experiments 1-4

Experiment 1

The objectives of this experiment were to: 1) select seedlings of the populations dwarf V. ashei 'W78-66' selfed and V. ashei 'Beckyblue' x 'Premier' that grew well in vitro. 2) clone the vigorous seedlings from each cross to be used in later experiments. On June 27, 1983 seeds from each cross were surface-sterilized with 30% Clorox (1.6% sodium hypochlorite) for 15 minutes, rinsed twice in sterile, deionized water and placed in vials containing 10ml of a 0.55% agar gel. The agar was autoclaved at 1.05 Kg/cm^2 for 15 minutes.

Ten seeds were placed in each vial, and 50 vials were used per cross. The vials were placed in front of a window to receive indirect sunlight 9 hrs per day. Cold requirements were met by placing the vials in an unlit refrigerator (7°C) 15 hrs each day for 30 days.

On September 27, 1983 vigorous seedlings from each cross were removed from the vials and cut into two-node cuttings. Cuttings were placed into vials containing 10 ml of modified Anderson's Rhododendron Medium (ARM) (Table 3-8) which had been previously autoclaved as described for the agar water. Vials were placed on a bench where they

Table 3-8. Composition of Modified Anderson's Rhododendron Medium (ARM).

Constituents	Concentration (mg/liter)
NH_4NO_3	400
KNO_3	480
$\text{CaCl}_2 \cdot 4\text{H}_2\text{O}$	440
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	370
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	380
Na_2EDTA	74.5
$\text{FeSO}_4 \cdot \text{H}_2\text{O}$	55.6
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	16.9
$\text{ZnSO}_4 \cdot \text{H}_2\text{O}$	8.6
H_3BO_3	6.2
$\text{Na}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$	.25
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	.025
KI	.83
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	.025
Thiamine HCl	.4
Myo-inositol	100
Adenine Sulfate	80
Casein Hydrolysate	1000
Sucrose	30,000
Agar	4,000
Zip	5

pH adjusted to 5.7 with 1N NaOH. Autoclaved at 1.05 Kg/cm^2 for 15 minutes.

received approximately 1 klux light provided by cool white fluorescent tubes for 15 hrs per day and were in the dark the other 9 hrs.

Internode lengths were measured from 5 clones of each cross on March 5, 1984. Five stems from each clone were measured. Two-node cuttings from each clone were then subcultured onto fresh modified ARM. Shoot number per vial, height of the tallest shoot, and internode measurements were collected for 3 clones from each cross on August 23, 1984. Clones were then subcultured by placing 2-node cuttings in vials of modified ARM.

Experiment 2

The objective of this experiment was to determine the effect of exogenously-applied GA on internode lengths of the dwarf and nondwarf clones growing in vitro. The experiment was initiated August 27, 1984. Two clones from each of the previously described populations were used. The 4 levels of GA₃ used were 1.0, 5.0, 10.0, and 100.0 mg/liter. Two-node cuttings of each genotype were placed in vials containing 10 ml of modified ARM amended with the GA₃. The GA₃ amended media was autoclaved as before, after it was dispensed into the vials. There were 10 replicates per treatment. Vials received approximately 1 klux fluorescent light 16 hrs daily and were kept at about 25°C. Colony size, height of the tallest shoot, and number of shoots per colony were collected October 31, 1984.

Experiment 3

The objective of this experiment was to determine if W78-66 was more tolerant than normal plants to higher than optimal levels of the

cytokinin 2IP (6-gamma-gamma-dimethyl-allyl amino purine). Two clones from the dwarf and 2 from the normal cultures developed in Experiment 2 were used, with 10 replicates per clone. Four levels of 2IP were used: 5, 10, 20, and 40 mg/liter. One two-node cutting was placed in each vial. Vials were given approximately 1 klux fluorescent light for 16 hours/day. Colony size, height of tallest shoot, and shoot number were recorded for all surviving vials after 2 months.

Experiment 4

The objective of this experiment was to compare the in vitro growth of W78-66 seedlings and normal V. ashei on a medium containing low levels of cytokinin. Two clones from the dwarf, 2 from the normal populations, and 3 levels of 2IP (0.0, 0.1, and 1.0 mg/l) were used. Two-node cuttings were placed in vials containing 10 ml of modified ARM with the appropriate level of 2IP. There were 8 replicates per treatment. Plants were treated as in other experiments. On March 21, 1985 height of tallest shoot, number of shoots, colony diameter, and internode length were recorded.

Results and Discussion

Experiment 1

Germination had begun in both populations by July 15, 1983, less than 3 weeks after sowing. After 2 months, most seeds from the cross V. ashei 'Beckyblue' x 'Premier' had germinated and produced tall, vigorous seedlings with a well-branched root system and large internode lengths. Seedlings from W78-66 selfed were much shorter than from the crossed

population; the shoots were vigorous with many short internodes, but they often fell over due to having a weak root system. Germination percentage was much reduced compared with seed from the 'Beckyblue' x 'Premier' cross. From approximately 100 seedlings, 7 seedlings with 3 cotyledons and 2 with variegated white and green cotyledons were found from this population.

Inbreeding depression may account for much of the poor germination with the dwarf selfed seeds. Selfing could also account for the 2 types of anomalous seedlings: tricotyledons and variegated cotyledons. Selfed seeds were used despite the inherent problems in order to obtain seedlings with the maximum expression of the dwarf trait.

Vigorous seedlings which appeared to have short internodes were selected from the dwarf selfed population for subculturing (Table 3-9). Seedlings of the other cross were not selected for internode length, but the ones used had internode lengths at least twice the size of the dwarf population (Figure 3-3).

Shoot number did not differ significantly among clones of the different populations (Table 3-10), but average height and internode length of all the shoots in a vial were about half as great in the dwarf populations as in the normal population (Table 3-10).

Experiment 2

Height increased significantly with increasing levels of GA_3 in the dwarf clone grown in vitro (Table 3-11). The nondwarf clone did not differ in height among the different levels of GA_3 (Table 3-11). Surprisingly, axillary shoot development increased significantly with increasing levels of GA_3 with both plant types. Cytokinins and their

Table 3-9. Internode length of 5 V. ashei dwarf clones (W78-66 selfed) and 5 normal V. ashei clones (Beckyblue x Premier) in vitro.

Clone	W78-66 (selfed) (cm)		Beckyblue X Premier (cm)	
	Mean	SD	Mean	SD
1	.10 a ^z	.08	.30 a ^z	.03
2	.12 a	.03	.46 a	.12
3	.12 a	.02	.34 a	.08
4	.15 a	.04	.23 a	.02
5	.15 a	.01	.21 a	.02
	$\bar{X} = .13b$	.03	.30 a	.11

^zMean separation within columns by Duncan's Multiple Range Test, 5% level.

Table 3-10. Growth of dwarf and control seedlings of V. ashei in vitro.

Clone	Shoots per vial (no.)	Height of tallest shoot (cm)	Internode length (cm)
W78-66 (selfed)			
Clone 2			
vial 1	27 a ^z	2.0b ^z	.16b ^z
vial 2	19 a	2.5b	.17b
Clone 3			
vial 1	27 a	3.0b	.16b
Clone 6			
vial 1	23 a	3.7b	.16b
$\bar{X} =$	24 a	2.8b	.16b
Beckyblue x Premier			
Clone 1			
vial 1	25 a	6.0 a	.42 a
vial 2	19 a	5.5 a	.33 a
Clone 4			
vial 1	26 a	4.0 ab	.25 a
vial 2	29 a	3.5b	.28 a
$\bar{X} =$	25 a	4.8 a	.32 a

^zMean separation within columns by Duncan's Multiple Range Test, 5% level



Figure 3-3. Growth of W78-66 selfed (right) and 'Bluebelle x 'Premier' (left) after 3 months in vitro.

Table 3-11. Growth of dwarf (W78-66 selfed) and nondwarf ('Beckyblue' x 'Premier') V. ashei clones in vitro as affected by GA₃ level.

GA ₃	Dwarf			Nondwarf		
	Plants (no.)	Height (cm)	Shoot (no.)	Plants (no.)	Height (cm)	Shoot (no.)
0 mg/liter	8	.75	30	10	4.2	6.5
1	9	.89	17	5	3.5	11.0
5	9	1.26	28	7	3.3	10.0
10	5	1.48	36	7	3.3	16.0
100	8	1.58	65	9	3.6	33.0
Significance ^z						
Genotype						
Linear		**	**		**	**
GA ₃ Level						
Linear		**	**		**	**
Quadratic		ns	ns		ns	ns
Cubic type		ns	ns		ns	ns

z**, * Significant at .01 and .05 level respectively, ns=not significant.

interaction with auxins are reported to stimulate axillary bud development in vitro (Murashige, 1974), while gibberellins are reported to repress axillary bud development. Axillary shoot development in potato can be controlled by manipulation of levels of auxin, gibberellin, and cytokinin (Kumar and Wareing, 1972 as cited in Wareing and Phillips, 1978).

Abnormal growth of the dwarf and nondwarf plants in vitro was observed only at the 2 highest levels of GA_3 . The effect was mainly on leaf appearance. Leaves from plants receiving the 2 highest levels of GA_3 (10 and 100 mg/liter) were sometimes long and narrow. This leaf type was found with both dwarf and normal genotypes.

Experiment 3

Height, shoot number per vial, and colony diameter were neither significantly different between the dwarf and nondwarf, nor did they change significantly with increasing levels of cytokinin (Table 3-12). Few replications due to high plant loss at the high cytokinin levels and large variability among replications are the probable reasons for the lack of significance. Trends seemed to be indicated, but this experiment was not sufficiently precise to show them clearly. Height tended to decrease with higher levels of 2IP in both dwarf and nondwarf plants. Shoot number and colony size appeared greatest with 10 mg/liter 2IP in the dwarf clones and decreased at higher levels. The nondwarf had its highest shoot number but lowest colony diameter at 20 mg/liter 2IP.

Cytokinin levels above 10 mg/liter appear to be detrimental to overall growth of both plant types. Based on this experience selection

Table 3-12. Growth of dwarf (W78-66 selfed) and nondwarf ('Beckyblue' x 'Premier') *V. ashei* clones in vitro as affected by high cytokinin levels.

2IP level	Vials (no.)	Survival (%)	Height (cm)	Shoot (no.)	Colony size ^z (cm)
Dwarf					
5 mg/liter	15	.75	1.1	15	1.9
10	5	.25	1.1	40	2.4
20	3	.15	.9	27	1.3
40	2	.10	.6	13	.3
Nondwarf					
5	10	.50	2.3	36	2.3
10	5	.25	1.1	38	1.7
20	4	.20	1.0	47	1.4
40	0	.00	---	--	---
Significance ^y					
Genotype					
Linear			ns	ns	ns
2IP level					
Linear			ns	ns	ns
Quadratic			ns	ns	ns
Cubic			ns	ns	ns

^zWidth x depth.

^yns=not significant.

for dwarfism using high levels of cytokinin (Lane and Looney, 1982) would not be an efficient method with the type of dwarfism exemplified by W78-66.

Experiment 4

The effect of cytokinin levels was not statistically different between the dwarf and nondwarf types in any of the parameters measured (Table 3-13). Plant height and shoot number had linear relationships with cytokinin levels. Height decreased and shoot number increased as cytokinin level increased up to 1.0 mg/liter 2IP. The colony diameter and internode length relationship was curvilinear. Internode length initially decreased and the number of shoots increased as cytokinin level increased. Colony size initially increased and then decreased with increasing cytokinin. While the pattern of growth was similar, the dwarf was shorter and had shorter internodes than the nondwarf. Consequently, dwarfness as exhibited in the W78-66 is probably not due to differences in endogenous cytokinin levels nor is the dwarf more sensitive to exogenously-applied cytokinin levels.

Inheritance of dwarfism in *V. ashei* W78-66.

Introduction

The objective of this research was to observe how the dwarfism in *V. ashei* W78-66 is inherited.

Table 3-13. Growth in vitro of dwarf (W78-66 selfed) and nondwarf ('Beckyblue' x 'Premier') V. ashei clones as affected by low cytokinin levels.

Genotype	2IP level (mg/liter)	Vials (no.)	Height (cm)	Shoots (no.)	Colony size ^z (cm)	Internode length (cm)
Dwarf						
	0.0	10	3.0	1.0	.5	.24
	0.1	10	2.9	3.0	.9	.22
	1.0	10	2.2	12.7	1.7	.15
Nondwarf						
	0.0	10	4.8	1.1	.6	.54
	0.1	10	4.7	1.5	1.0	.43
	1.0	10	3.7	13.0	2.0	.33
Significance ^y						
Genotype						
Linear			**	ns	ns	**
2IP level						
Linear			**	**	**	**
Quadratic			ns	ns	**	**

^zlength x width.

^y** , * Significant at .01 and .05 level, respectively, ns=not significant.

Materials and Methods

During the spring of 1979 cross-pollinations were made by Dr. P.M. Lyrene, between the dwarf V. ashei (W78-66) and commercial cultivars of V. ashei. In preparation for crossing, plants were placed in a refrigerated room at 4°C for 30 days after being dug from the field to insure that the cold requirement had been met. The dwarf plant was used as the female parent, and pollen from 4 V. ashei cultivars was bulked and applied to depetaled flowers before anthesis in the greenhouse. Berries were collected as they ripened and seed was extracted using a food blender. Dried seed was stored at 4°C for 5 months.

The seed was sown on moist, sterilized peat moss in the greenhouse on November 7, 1980 and most germinated within 120 days. The seedlings were transferred to flats of peat moss. After 4 months the plants were transplanted from the greenhouse to a high-density field nursery. Plant spacing was 25 cm between rows and 12 cm within the row. Plants were fertilized and irrigated as required.

W78-66 was self-pollinated in the greenhouse during the spring of 1980. The resulting seeds and seedlings were treated as before.

Plants were selected from the F_1 population December, 1983 for use in further crosses. Selected plants had relatively short internodes compared with the rest of the population. Cross-pollinations were made between the short internode F_1 's; backcrosses to both parents were made and W78-66 was self-pollinated (Table 3-14). Berries were harvested when ripe. Ten berries from each plant were weighed and number of plump seeds per berry were counted. Seeds were removed from the remaining berries, and all seeds were dried and treated as before. Seedlings were planted into a high density nursery May 23, 1984.

Table 3-14. Pollination records, Spring 1983.

Population	Pollination period	Pollinations (no.)	Harvest duration	Fruit dev. period ^z (days)	Fruit harvested (no.)	Fruit set (%)	Plump seed per fruit (no.)
(W78-66 x tall cultivar) x (W78-66 x tall cultivar) (Both parents selected for short internodes)							
82-197 x 82-200	2/22-3/17	189	5/25-7/25	102	66	35	16
82-200 x 82-199	3/01-3/17	89	5/16-7/25	81	89	100	21
82-199 x 82-166	3/01-3/08	80	7/25	147	1	3	2
82-166 x 82-201	2/07-3/21	215	5/16-7/10	93	36	17	9
82-201 x 82-197	2/22-3/17	143	5/20-6/28	99	30	21	23
82-157 x 82-158	2/08-2/21	185	5/11-5/16	87	3	2	31
82-158 x 82-160	2/10-2/21	289	5/01-6/20	88	97	33	40
82-160 x 82-161	2/12-3/01	172	5/16-5/25	91	7	4	26
82-161 x 82-156	2/12-3/16	275	5/16-7/05	99	224	81	40
82-156 x 82-157	2/12-2/19	250	4/26-6/04	80	137	55	33
Tall cultivar x (W78-66 x tall cultivar) (Pollen parent selected for short internodes)							
Aliceblue x 82-167	2/28-3/16	55	5/11-7/05	79	55	100	59
Bonita x 82-201	2/09-2/28	457	4/30-6/08	82	59	13	48
Bluebelle x 82-166	2/14-3/03	442	5/16-7/25	100	367	75	50
(W78-66 x tall cultivar) x W78-66 (Female parent selected for short internodes)							
82-194 x W78-66	2/24-3/22	165	5/16-7/19	91	156	98	33
82-195 x W78-66	2/27-3/25	176	5/25-7/10	94	160	90	13
82-196 x W78-66	2/27-3/25	155	6/01-7/25	100	94	62	29
Tall cultivar x W78-66							
Bonita x W78-66	3/29-4/04	422	4/30-6/08	82	8	2	33
Aliceblue x W78-66	2/24-2/27	55	5/01-6/01	86	43	78	32
W78-66 selfed	2/24-3-11	565	6/01-7/19	91	178	31	28

^zInterval between 50% pollination and 50% fruit harvest.

Height, stem diameter, and internode length were measured on the 1980, F_1 population and the 1981, selfed population during September 1983. Height, number of shoots per plant, and internode length were measured on the 1983 populations during September, 1984. Plant height was measured as the height of the tallest stem. Internode length was measured on newly mature wood of the tallest shoot averaged over 4-5 internodes.

For comparison, measurements were collected on 2 nondwarf hybrid V. ashei populations and on populations obtained by selfing 2 tall V. ashei cultivars. Six nondwarf hybrid populations were also measured in the 1983 high-density nursery.

Results and Discussion

The F_1 population from W78-66 x 6x V. ashei cultivar composite averaged significantly shorter and had shorter internodes than one of the tall V. ashei x tall V. ashei populations (79-18 x 79 QP), but had about the same height and internode length as the other (79-17 x 79 EP). (Table 3-15). Stem diameter did not differ significantly in the 3 populations.

The W78-66 selfed population averaged significantly smaller in all parameters measured than both check populations ('Climax' x 'Beckyblue' and 'Climax' x NC1830) and the other selfed population ('Premier' selfed).

The 3 hybrid populations ('Climax' x 'Beckyblue', 'Climax' x NC1830, and 'Premier' x V. ashei Late Composite) were compared with the 2 selfed populations (W78-66 selfed and 'Premier' selfed) to distinguish the effects of crossing vs selfing vs dwarfism. Height was

Table 3-15. Growth of seedling populations of V. ashei as affected by population, age, and dwarfism.

Family	Plant Type ^z	(no.)	Age	Height (cm)			Internode length (cm)			Stem diameter (mm)		
				Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
W78-66 x Comp. ^y	1	117	2yr	74.6b ^x	22.3	30-134	1.0b ^x	.25	.55-1.7	9.4a ^x	2.9	3.5-18.5
79-17 x 79EP ^w	2	33	"	86.4ab	22.3	50-138	1.0b	.26	.50-1.7	10.4a	2.8	4.5-18.0
79-18 x 79EP ^w	2	33	"	99.5a	24.7	42-140	1.5a	.34	1.00-2.0	10.5a	2.8	4.5-16.0
Premier selfed	3	107	"	73.5b	30.5	14-132	1.1b	.26	.50-1.8	6.3b	2.8	2.0-13.0
Climax x Beckyblue	2	33	1yr	84.4a ^x	20.2	46-129	1.1a ^x	.27	.70-1.9	8.1a ^x	1.8	5.0-12.0
Climax x NC1830 ^w	2	33	"	93.0a	14.0	61-124	1.2a	.31	.60-1.8	8.0a	1.6	5.0-10.5
W78-66 selfed	4	114	"	31.5c	15.3	6-90	.6b	.19	.20-1.1	4.1b	1.6	1.0- 8.0
Premier selfed ^y	3	114	"	61.6b	29.9	5-117	—	—	—	4.3b	1.8	0.9- 8.4
Premier x Comp ^y	2	82	"	95.5a	31.5	15-161	—	—	—	7.3a	2.7	1.5-13.6

^zType 1= dwarf x tall, 2=tall x tall, 3= tall selfed, 4=dwarf selfed.

^yComposite of pollen from 4 V. ashei cultivars.

^xMean separation within columns within groups by Duncan's Multiple Range Test, 5% level.

^w79 EP and 79 QP are V. ashei composites of select clones from the cultivar development program. NC1830 is a fertile hexaploid V. ashei x V. constablaei hybrid.

^yData from another experiment and included for comparison only, internode data not collected.

significantly less in the W78-66 selfed population than in the 'Premier' selfed which was less than in the 3 crossed populations. These measurements indicated that both inbreeding depression and short-stature genes from W78-66 contributed to the dwarfism of the W78-66 selfed progeny. Internode length was reduced in the W78-66 selfed population compared with the 2 crossed populations ('Climax' x 'Beckyblue' and 'Climax' x NC1830). Inbreeding depression did not greatly affect internode length based on comparisons among 2-year old populations (Table 3-15). Therefore the reduction in internode length in the 1-year old W78-66 selfed population compared to the 2 crossed populations was probably due mainly to dwarfism.

Stem diameter was much smaller in all selfed populations than in hybrid populations. Stem diameter did not differ statistically between the 2 selfed populations (W78-66 selfed and 'Premier' selfed) of the same age. Reduced stem diameter of the W78-66 selfed population probably measures inbreeding depression, not effects of dwarfism.

The standard deviations for height and internode lengths were relatively low for the W78-66 selfed population compared to the other populations (Table 3-15) which suggests that W78-66 is not segregating for a major gene affecting height and internode length.

High correlations were found between stem diameter and height in most populations (Table 3-16). A low, nonsignificant correlation was measured in the 'Climax' x NC 1830 population, which may be due to the interspecific hybrid nature of the NC 1830 parent (V. constablaei x V. ashei 'Premier').

Significant correlations between height and internode length were seen only in one hybrid population (79-18 x 79QP and in the W78-66-

Table 3-16. Correlations between height, stem diameter, and internode length of different V. ashei populations measured September, 1983.

Population	Plants			Stem diameter x Height	Stem diameter x Internode length	Height x Internode length
	Type	(no.)	Age			
W78-66 x 6x Cv Comp	1	117	2yrs	.77**	.11ns	.14ns
79-17 x 79 EP	2	33	"	.67**	.21ns	.24ns
79-18 x 79 OP	2	33	"	.40**	.18ns	.46**
Premier selfed	3	107	"	—	—	—
Climax x Beckyblue	2	33	1yr	.47**	.17ns	.16ns
Climax x NC1830	2	33	"	.17ns	-.23ns	.02ns
W78-66 selfed	4	114	"	.55**	.21ns	.30**
Premier selfed	3	114	"	.32**	—	—
Premier x Late Comp	2	82	"	.87**	—	—

^z1=dwarf x tall; 2= tall x tall; 3= tall selfed; 4= dwarf selfed.

** * Significant at .01 and .05 level respectively, ns=not significant.

selfed population. Height of a plant is determined by the number of nodes on the stem and the internode length.

Plant height measurements in the 1984 high density nursery were greatest in populations not having W78-66 in their pedigree and least in the (W78-66 x tall cultivar) x W78-66 population (Table 3-17). The one exception was in the cross (79-69 x 83-17), in which a new dwarf phenotype was found to be segregating, with about 15% of the seedlings distinctly dwarfed. The parents of this cross were both tall wild V. ashei clones, one of which came from Baldwin, FL (78-69) and the other from south Georgia (83-17).

The populations having the most consanguinity with W78-66 had, in general more shoots per plant, but there were some exceptions to this trend. Shoot number and plant height were interrelated and the nature of this association was different in the dwarf and nondwarf populations. This association is discussed further in a later section.

Populations with W78-66 in their pedigrees generally had shorter internodes than populations not related to W78-66 (Table 3-18). The second check population, in which a new dwarf plant type was segregating, was an exception to this pattern.

Scatter diagrams (Appendix 1 Fig. 1 through 14) show the relationship between height and internode length in each of the 14 populations. In most cases, plant height was positively correlated with internode length and many populations had similar r values, but the nature of the relationship varied among populations.

The largest height by internode length correlation coefficients were found in populations from selected dwarf F-1 plants backcrossed to W78-66 (Table 3-18). The lowest r values were found in 2 of the 4

Table 3-17. Growth of dwarf and nondwarf populations of *V. ashei*.

Population	Seedlings (no.)	Height (cm)			Shoots (no.)			Internode length (cm)		
		Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Populations not having W78-66 in the pedigree										
78-41 x 83-4	79	40.1a ^z	10.6	10-60	18b ^z	7.3	4-40	1.3b ^z	.26	.8-2.1
78-69 x 83-17	79	35.1c	11.0	8-60	18b	9.3	5-54	1.1c	.24	.6-1.8
83-44 x 83-24	80	41.0a	12.3	13-71	14c	7.7	3-46	1.3b	.32	.8-2.1
83-45 x 83-19	80	39.8ab	12.4	8-64	11d	5.3	2-28	1.5a	.28	.6-2.5
Populations having W78-66 in the pedigree										
Tall cultivar x (W78-66 x tall cultivar)										
Aliceblue x 82-167	80	36.7bc	11.0	15-64	15c	6.8	4-45	1.3b	.29	.4-1.8
Bonita x 82-201	80	34.6c	10.1	8-58	14c	6.0	3-31	1.1c	.20	.5-1.5
Bluebelle x 82-166	80	36.6bc	10.4	9-72	20a	11.7	5-84	1.1c	.23	.6-1.8
Tall cultivar x W78-66										
Bluebelle x W78-66	82	36.2c	11.5	11-56	21a	11.2	4-54	1.1c	.21	.7-1.6
(W78-66 x tall cultivar) x (W78-66 x tall cultivar)										
82-201 x 82-197	94	31.1d	8.7	7-52	19ab	8.6	4-54	1.0cd	.17	.5-1.4
82-158 x 82-160	170	21.8f	8.5	4-45	19ab	11.4	1-76	1.0cd	.30	.3-1.7
82-160 x 82-161	92	23.2f	10.9	4-46	18b	9.1	3-45	.9d	.28	.3-1.6
82-161 x 82-156	80	30.1de	10.2	7-49	20a	11.0	3-66	1.1c	.22	.4-1.6
(W78-66 x tall cultivar) x W78-66										
82-195 x W78-66	80	22.3f	10.3	3-68	20a	11.0	6-61	.8d	.22	.3-1.4
82-196 x W78-66	94	27.4e	9.2	6-52	19ab	9.8	4-54	.9d	.20	.4-1.5

^zMean separation within columns by Duncan's Multiple Range Test, 5% level.

Table 3-18. Correlations between height, shoot number and internode length in various V. ashei populations, measured September, 1984.

Population	Seedlings (no.)	Height vs Shoot number	Height vs Internode length	Internode length vs Shoot number
Populations not having W78-66 in the pedigree				
78-41 x 83-4	79	.25*	.21*	.09ns
78-69 x 83-17	79	.22*	.25*	-.19ns
83-44 x 83-24	80	.37**	-.03ns	-.04ns
83-45 x 83-19	80	.33**	.01ns	-.11ns
Populations having W78-66 in the pedigree				
Tall cultivar x (W78-66 x tall cultivar)				
Aliceblue x 82-167	80	.16*	.36**	-.08ns
Bonita x 82-201	80	.16ns	.31**	.03ns
Bluebelle x 82-166	80	.06ns	.34**	-.06ns
Tall cultivar x W78-66				
Bluebelle x W78-66	82	.28**	.22**	-.05
(W78-66 x tall cultivar) x (W78-66 x tall cultivar)				
82-201 x 82-197	94	.31**	.32**	.02*
82-158 x 82-160	170	-.11ns	.35**	-.01ns
82-160 x 82-161	92	.14ns	.24**	-.11ns
82-161 x 82-156	80	.26**	.30**	.04ns
(W78-66 x tall cultivar) x W78-66				
82-195 x W78-66	80	.03ns	.55**	-.17ns
82-196 x W78-66	94	.25**	.49**	-.14ns

**, * Significant at .01 and .05 level, respectively, ns=not significant.

populations which did not have W78-66 anywhere in their pedigrees. Moderate but significant r values were found in the remaining populations.

Scatter diagrams also show the relationship between height and number of shoots in the various populations (Appendix 1 Figure 15 through 28). Most significant correlations between these 2 variables are found in the populations which do not have W78-66 in their pedigrees, but all are below .37.

In certain F_2 populations resulting from crossing selected short internode F_1 plants, both populations resulting from backcrossing short internode F_1 's to W78-66, and the W78-66 selfed population, a unique plant phenotype segregated (Table 3-19 and Figure 4). These plants were all less than 10 cm tall and had at least 25 extremely compact branches and internode lengths of 4 mm or less. Some of these plants appeared to be quite vigorous, but they were far shorter than W78-66 or any of the highly-tillered F_1 plants obtained by crossing W78-66 x tall cultivars.

Inheritance patterns in polyploids can be complex and depend upon the behavior of the chromosomes that carry the genes responsible for the trait being studied, and the number and nature of the different alleles present at the different loci (Burnham, 1962). Chromosomes may range from being completely homologous with the corresponding chromosomes in the different genomes to totally lacking homology in corresponding chromosomes. Most natural polyploids range in chromosome homology within the genome. This would imply that disomic, polysomic, and intermediate ratios for different characters are possible in progenies from the same plant (Hermesen, 1984).

Table 3-19. Segregation ratios of the extreme dwarf phenotype in different populations of V. ashei.

Population	Seedlings (no.)	Extreme dwarf (no.)	Ratio of extreme dwarf	Ratio of tall plants
Populations not having W78-66 in pedigree				
78-41 x 83-4	79	0	—	y
78-69 x 83-17	79	0	—	y
83-44 x 83-24	80	0	—	y
83-45 x 83-19	80	0	—	y
Populations having W78-66 in the pedigree				
Tall cultivars x (W78-66 x tall cultivars)				
Aliceblue x 82-167	80	0	—	y
Bonita x 82-201	80	0	—	y
Bluebelle x 82-166	80	0	—	y
Tall cultivars x W78-66				
Bluebelle x W78-66	82	0	—	y
(tall cultivars x W78-66) x (tall cultivars x W78-66)				
82-201 x 82-197	94	0	—	y
82-158 x 82-160	170	13	1:13	y
82-160 x 82-161	92	8	1:11.5	y
82-161 x 82-156	80	1	1:80	y
Total	342	22	1:15.5	
(tall cultivars x W78-66) x W78-66				
82-195 x W78-66	80	12	1:6.7	y
82-196 x W78-66	94	3	1:31	y
Total	174	15	1:11.6	
W78-66 selfed ^z	76	14	y	1:5.4

^zPlants are in a different location and 1 year younger than plants from the other populations listed.

^yRatio could not be determined due to lack of clear distinction between classes.

Figure 3-4. Progeny from crosses with and without W78-66 in the pedigree. 4-a. Progeny of population without W78-66 in the pedigree. 4-b, c. Progeny of populations with W78-66 in the pedigree, ex=extreme dwarf, t=tall.



Since V. ashei is hexaploid, it could exhibit disomic, tetrasomic, and/or hexasomic inheritance in the dwarf trait. It is also possible in tetrasomic or hexasomic inheritance to have nonrandom chromosome pairing at meiosis which would alter segregation ratios. This was reported in the tetraploid V. australe Small (Draper and Scott, 1971). Since some plants in the F_1 populations (W78-66 x tall cultivar) and backcross to tall cultivars [(W78-66 x tall cultivars) x tall cultivars] have dwarf characteristics, the dwarf trait is somewhat dominant in its expression and is possibly controlled by 1 or 2 genes. If dwarfism as expressed in W78-66 is controlled by only 1 or 2 genes, the extreme dwarf found in many of the populations resulting from a cross between 2 short-internode F_1 's, when a short-internode F_1 is backcrossed to a tall cultivar, and when W78-66 is selfed, may be homozygous for the dwarf trait, and therefore W78-66 would be heterozygous for the trait. Since dwarfs have not been found in crosses between V. ashei cultivars (Lyrene, per. comm.), they are probably homozygous for normal height. More crosses need to be made, such as the continued backcrossing of short-internode segregates with tall cultivars, and crossing of the extreme dwarf segregates with W78-66, tall cultivars, and selfing before the inheritance pattern is better understood.

SECTION IV

SELF-INCOMPATIBILITY IN VACCINIUM ASHEI (READE)

Introduction

The objectives of this research were to measure and describe self-incompatibility in native Vaccinium ashei plants selected from north Florida and south Georgia.

Materials and Methods

In spring 1984, 19 rabbiteye blueberry plants from throughout north Florida and south Georgia were self- and cross-pollinated (Table 4-1). These plants had been selected during the previous 5-year period, primarily on the basis of large fruit size and high fruit quality. The plants had been propagated by cuttings, grown to flowering size in a field nursery, and then grown in 12-liter pots for a year before they were used in this study. In January, 1984, the plants were placed in a refrigerator at 7°C for 4 weeks to satisfy their chilling requirement, and then were moved to a greenhouse maintained between 4°C and 25°C for flowering. Each plant was divided into 3 parts: 2 sections were self-pollinated and 1 section was cross-pollinated. Approximately 200 flowers of each section were used. Both crossed and selfed flowers were emasculated. The pollen used in cross-pollination of all native plants was a bulk collected from the 4 V. ashei cultivars 'Bonita', 'Bluebelle', 'Climax', and 'Beckyblue'.

Table 4-1. Location of the 19
native V. ashei clones.

Clone	County, State
W78-72	Columbia, FL
W78-112	Okaloosa, FL
W78-77	Escambia, FL
W78-74	Okaloosa, FL
W78-39	Washington, FL
83-42	Nassau, FL
83-44	Washington, FL
83-40	Nassau, FL
83-43	Washington, FL
W78-102	Okaloosa, FL
W78-34	Leon, FL
83-47	Okaloosa, FL
W78-41	Okaloosa, FL
W78-40	Okaloosa, FL
83-46	Okaloosa, FL
83-45	Okaloosa, FL
83-41	Ware, GA
W78-69	Nassau, FL
W78-66	Nassau, FL

Male fertility of the 19 native clones was measured by pollen germination using the "hanging drop technique" (Stanley and Linskens, 1964) and fruit set after pollination of the two cultivars 'Bluegem' and 'Climax'. Germination medium was that of Goldy and Lyrene (1983) and consisted of 100mg/liter H_3BO_3 , 300mg/liter $Ca(NO_3)_2 \cdot H_2O$, 200 mg/liter $MgSO_4 \cdot 7 H_2O$, 100mg/liter KNO_3 , and 10% sucrose. Female fertility was measured when native plants were cross-pollinated with bulked cultivar pollen.

Seven of the native V. ashei clones were used to study pollen germination and pollen tube growth after cross- and self-pollination. For this study 5 styles were collected from each of the 7 clones at each of 6 intervals after pollination (12, 24, 36, 48, 72, and 96 hr) for both cross- and self-pollinations. Styles were stained with water-soluble Aniline blue and examined by fluorescence microscopy (Kho and Baer, 1968).

Fruit set, fruit weight (10 berries/treatment), number of seedlings per fruit, number of germinated seeds/pollination, fruit development period (interval of 50% pollination to 50% ripe fruit), and harvest duration were recorded. Self-fruitfulness and self-fertility were calculated for each clone.

Self-fruitfulness was defined as the number of fruit per pollination when self-pollinated, divided by the number of fruit per pollination when cross-pollinated. Self-fertility was measured by 1) the number of seedlings produced per fruit when selfed, divided by the number of seedlings produced per fruit when crossed, and 2) the number of seedlings produced per pollination when selfed divided by the number of seedlings produced per pollination when crossed.

Results and Discussion

Pollen germination varied from 8% to 73% among native plants, but there was considerable variation in different samples from the same clone (Table 4-2). Pollen fertility, measured as the percent fruit set after pollination onto V. ashei cultivars, varied from 29% to 89% among the 19 native V. ashei clones. The low pollen germination of 83-42 (8%) was not reflected in the pollination test, and females self-pollinated set well. Fruit set varied from 3% to 82% for the 19 V. ashei clones when they were pollinated with a bulk of pollen from 4 V. ashei cultivars (Table 4-2).

While pollen had germinated on the stigmas of all plants by the end of 12 hr, its rate of growth in the style was highly variable (Table 4-3). All seven plants were not pollinated on the same day and the slow growth of the pollen tubes of 3 plants 78-72, 78-77, and 78-112 may have been due to low temperatures after pollination of these plants (Knight and Scott, 1964).

Self-fruitfulness as measured by percent fruit set after selfing divided by percent set after crossing varied from 0.0% to 68% (Table 4-4). Five native plants had a relative fruit set greater than 50%, 9 were greater than 20%, and only 1 plant was completely self-unfruitful.

Relative self-fertility compared to cross-fertility (based on number of seedlings/fruit after selfing divided by seeds per fruit after crossing) varied from 0% to 110% (Table 4-4). Relative self-fertility as the number of seedlings/pollination varied from 1% to 45% (Table 4-4).

Fruit set was not correlated with number of seed per fruit, indicating that plants like 83-45, which have a high fruit set but few

Table 4-2. Fertility of 19 clones of V. ashei from south Georgia and north Florida measured by pollen germination on an artificial medium, fruit set after pollination of known fertile plants, and fruit set after cross-pollination.

Clone	Pollen germination ^z		Fruit set when pollen put onto <u>V. ashei</u> cultivars ^y	Fruit set when pollinated by cultivars ^x
	Mean	Range	(%)	(%)
W78-72	73	53-90	89	78
W78-112	22	13-29	72	3
W78-77	22	14-29	56	31
W78-74	39	34-50	38	65
W78-39	73	54-77	66	82
83-42	08	05-14	53	47
83-44	41	27-52	67	62
83-40	40	26-53	61	77
83-43	43	15-76	56	75
W78-102	33	21-43	77	81
W78-34	43	20-65	53	45
83-47	59	52-68	57	60
W78-41	36	25-52	29	67
W78-40	35	16-57	38	40
83-46	57	40-77	37	55
83-45	33	27-39	58	45
83-41	66	30-85	76	52
W78-69	62	52-75	33	76
W78-66	23	10-39	63	52

^z10% Sucrose - .01% boron solution for 4 hours. Five samples were tested per clone.

^yNumber of fruit/pollination on V. ashei cultivars when the native plants were the pollen parents. Approximately 30 flowers pollinated to test each clone.

^xNumber of fruit/pollination when native plants were pollinated with a composite pollen from V. ashei cultivars. Approximately 200 flowers were pollinated to test each clone.

Table 4-3. Hours required for pollen germination and growth in cross and self-pollinated pistils of 7 native V. ashei plants.

Clone	Pollen germination on stigma		Pollen tubes mid-way in style		Pollen tubes in ovary	
	crossed	selfed	crossed	selfed	crossed	selfed
83-42	12 hr	12 hr	36 hr	36 hr	48 hr	48 hr
W78-39	12	12	24	24	72	72
W78-40	12	12	36	36	48	72
W78-66	12	12	48	48	72	72
W78-72	12	12	48	48	>96	>96
W78-77	12	12	48	36	>96	>96
W78-112	12	12	72	72	>96	>96

Table 4-4. Percent self-fruitfulness and percent self-fertility of 19 native plants of V. ashei from south Georgia and north Florida.

Clone	Self-fruitfulness (%)		Self-fertility	Self-fertility
	actual ^z	relative ^y	sf/sf ^x (%)	sp/sp ^w (%)
W78-72	ob ^v	0c ^v	0c ^v	0 ^b
W78-112	#	#	#	#
W78-77	2b	6c	60ab	4b
W78-74	3b	5c	59ab	2b
W78-39	3b	1c	33b	1b
83-42	3b	6c	33b	2b
83-44	3b	8bc	33b	1b
83-40	6b	7bc	110a	7b
83-43	9b	12bc	32b	6b
W78-102	13b	16abc	15b	3b
W78-34	16ab	37abc	12b	4b
83-47	19ab	31abc	29b	10b
W78-41	20ab	30abc	20b	7b
W78-40	24a	60ab	33b	20a
83-46	29a	51abc	10b	6b
83-45	31a	68a	10b	7b
83-41	35a	66a	44b	6b
W78-69	35a	46abc	11b	7b
W78-66	36a	69a	23b	7b
Significance ^u				
Clone	*	*	**	*

Not calculated due to the high level of female sterility of the clone.

^z Percent fruit set when self-pollinated.

^y Number of fruit per pollination when selfed/number of fruit per pollination when crossed.

^x Number of seedlings per fruit when selfed/number of seedlings per fruit when crossed.

^w Number of seedlings per pollination when selfed/number of seedlings per pollination when crossed.

^v Mean separation within columns by Duncan's Multiple Range Test, 5% level.

^u **, *, significant at .01 and .05 level respectively, ns=not significant.

viable seed per fruit, may have a greater ability to develop parthenocarpic fruit than plants like 83-40, which had many viable seed/berry. Increasing the number of seed/berry increased fruit size and decreased the fruit development period (4-5).

In general, selfing resulted in reduced fruit set, smaller berries, fewer seed/fruit, fewer seed/pollination, and a longer fruit development period, but did not alter the duration of harvest (Table 4-6).

These results indicate that there is considerable variation for self-compatibility in the native Florida rabbiteye germplasm. The low level of self-compatibility in the present rabbiteye cultivars (El-Agamy, 1979) may be attributed to chance selection of high self-incompatibility in the 6 original plants (Lyrene, 1983). Self-compatibility has been transferred in other crops by making the appropriate crosses with compatible varieties (Ronald and Asher, 1975; Odland and Noll, 1950) and may be possible with V. ashei.

Although there was considerable variation in self-fruitfulness among the 19 native V. ashei clones tested, none would be successful horticulturally if planted in solid blocks. In general, levels of self-incompatibility in V. ashei appear substantially higher than in the V. corymbosum cultivars that have been tested (El-Agamy, 1979; Meader and Darrow, 1947; and Johnston, 1940).

Table 4-5. Fruit characteristics of 19 native rabbiteye blueberry plants from south Georgia and north Florida as affected by self and cross-pollination.

Clone	Fruit set (no.)	Fruit weight (g)	Seedlings per fruit (no.)	Seedlings per pol. (no.)	Fruit dev. ^z (days)	Harvest duration (days)
W78-72						
selfed	0	----	--	0.0	---	----
crossed (female) ^y	78	1.20	10	8.0	93	10.8
crossed (male) ^x	89	1.50	31	28.0	81	20.4
W78-112						
selfed	1	1.00	17	0.1	110	----
crossed (female)	3	.80	8	0.2	101	11.5
crossed (male)	72	1.80	18	12.0	95	21.3
W78-77						
selfed	2	.42	4	0.1	114	15.3
crossed (female)	31	1.00	6	2.0	100	17.4
crossed (male)	56	1.70	34	11.0	90	12.3
W78-74						
selfed	3	.60	3	0.1	116	14.8
crossed (female)	65	1.10	5	3.0	92	13.1
crossed (male)	38	1.50	34	20.0	104	21.6
W78-39						
selfed	3	.60	3	0.1	121	14.3
crossed (female)	82	1.20	9	7.0	94	13.3
crossed (male)	66	1.80	52	50.0	88	14.7
83-42						
selfed	3	.40	5	0.2	90	5.9
crossed (female)	47	.80	15	7.0	84	5.9
crossed (male)	53	1.80	21	10.0	91	14.5
83-44						
selfed	3	.80	4	0.1	103	7.9
crossed (female)	62	1.30	12	7.0	93	11.9
crossed (male)	67	1.70	24	17.0	85	16.3
83-40						
selfed	6	1.10	10	0.5	82	10.5
crossed (female)	77	1.60	9	7.0	80	6.5
crossed (male)	61	1.80	12	9.0	91	10.7
83-43						
selfed	9	.47	2	0.2	126	15.7
crossed (female)	75	1.10	5	4.0	85	22.5
crossed (male)	56	1.70	24	11.0	92	11.3
W78-102						
selfed	13	.40	2	0.3	102	12.6
crossed (female)	81	.80	13	11.0	105	15.2
crossed (male)	77	1.80	39	30.0	100	22.2
W78-34						
selfed	16	.56	1	0.2	101	15.3
crossed (female)	45	1.10	10	5.0	86	9.5
crossed (male)	53	1.80	30	17.0	88	11.5

Table 4-5. (continued)

Clone	Fruit set (no.)	Fruit weight (g)	Seedlings per fruit (no.)	Seedlings per pol. (no.)	Fruit dev. ^z (days)	Harvest duration (days)
83-47						
selfed	19	.85	3	0.6	124	15.4
crossed (female)	60	1.00	11	6.0	97	16.8
crossed (male)	57	2.10	30	16.0	81	11.8
W78-41						
selfed	20	.53	2	0.3	126	17.8
crossed (female)	67	1.10	6	4.0	102	23.1
crossed (male)	29	1.60	12	11.0	84	13.4
W78-40						
selfed	24	1.10	5	1.0	102	13.9
crossed (female)	40	1.50	13	5.0	99	15.5
crossed (male)	38	1.40	13	5.0	101	13.0
83-46						
selfed	29	.80	3	0.7	89	11.9
crossed (female)	55	1.20	23	13.0	81	13.9
crossed (male)	37	1.70	22	8.0	95	9.1
83-45						
selfed	31	.80	2	0.5	114	12.8
crossed (female)	45	.85	16	7.0	84	7.9
crossed (male)	58	2.00	40	34.0	82	11.8
83-41						
selfed	35	.80	7	0.5	80	11.3
crossed (female)	52	.70	16	8.0	82	7.2
crossed (male)	76	1.70	20	14.0	106	22.7
W78-69						
selfed	35	.90	1	0.4	100	15.8
crossed (female)	76	1.30	8	6.0	81	14.1
crossed (male)	33	1.50	21	8.0	81	14.1
W78-66						
selfed	36	.50	4	0.6	102	10.4
crossed (female)	52	1.10	17	9.0	99	8.4
crossed (male)	63	1.50	25	12.0	90	12.5

^zInterval between 50% pollination and 50% fruit harvest.

^yCharacteristics of 78-72 when cross-pollinated by V. ashei cultivars.

^xCharacteristics of V. ashei cultivar when pollinated by 78-72.

Table 4-6. Average fruit characteristics of 19 native rabbiteye blueberry plants as affected by self and cross-pollination.

Treatment	Fruit set (%)	Fruit weight (gm)	Seedlings per fruit (no.)	Seedlings per pol. development (no.)	Fruit development ^z (days)	Harvest duration (days)
Selfed	15b ^u	0.7c ^u	4c ^u	0.5c ^u	106a ^u	13a ^u
Crossed(female) ^y	58a	1.1b	11b	6.0b	92b	13a
Crossed (male) ^x	57a	1.7a	26a	17.0a	91b	15a
Significance ^w						
Self vs Cross ^y	**	**	**	**	**	ns
Self vs Cross ^x	**	**	**	**	**	ns
Cross ^y vs Cross ^x	ns	**	**	**	**	ns
Self vs Crossed ^v	**	**	**	**	**	ns

^zInterval between 50% pollination and 50% fruit harvest.

^yBulk pollen from V. ashei cultivars used to pollinate native V. ashei clones.

^xPollen from native V. ashei clones used to pollinate V. ashei cultivars.

^w**, *, significant at .01 and .05 level respectively, ns=not significant

^vCombined results from both types of cross pollination.

^uMean separation within columns by Duncan's Multiple Range Test, 5% level.

SECTION V

FEMALE STERILITY IN VACCINIUM ASHEI (READE)

Introduction

On September 23, 1978, cuttings from W78-122, a very large, vigorous blueberry plant, were collected in an abandoned rabbiteye plantation 5 km north of Gainesville, Florida. The original planting is believed to have been made between 1920 and 1930 with native seedlings from west Florida (Lyrene and Sherman, 1979). W78-122 had unusually large leaves and set no fruit even though it had flowered heavily. The objectives of this experiment were to study the nature of this large-statured, female-sterile plant.

Materials and Methods

Phenotype of W78-122 in its native environment

On August 10, 1983, height, trunk diameter (at 1 meter height), and number of large trunks/plant were collected from 11 of the largest plants (including W78-122) growing within 50m of W78-122. Ten fully-expanded leaves from 20 large rabbiteye plants growing in the same area were collected, and leaf area and leaf index (length/width) were measured. Leaf area was measured using a LiCor Portable Area Meter Model 3000. Presence or absence of stalked glands on the undersurface of the leaves was noted.

Phenotype at the Horticultural Research Unit

On August 17, 1983, 10 leaves from each of 7 random Vaccinium plants and W78-122 growing in the Germplasm Block at the Horticultural Research Unit were collected, and leaf area was measured. Ten leaves from each of 2 plants for each of 4 cultivars of V. ashei were collected at the same time, and leaf area was measured.

Meiotic study and chromosome counts

Meiosis was studied using pollen mother cells from developing inflorescence buds. Developing inflorescence buds were collected and fixed in 3:1 absolute ethanol:glacial acetic acid for 24 hr and stored in 70% ethanol until used. Anthers were removed from 4-5 flowers of an inflorescence, squashed in 1% acetocarmine stain, destained in 45% acetic acid, and viewed under phase contrast at 1000X.

Male fertility

Male fertility was measured as the percent pollen germination in an artificial medium (as described in Section 4) and percent fruit set of known fertile V. ashei cultivars when pollinated with W78-122 pollen. Both measurements were compared with results from a number of other native V. ashei plants.

Pollen germination and growth in pistil

The method used to study pollen germination and growth in the pistil of W78-122 and the other native plants was the same as that described in Section 4 of this paper.

Ovary and ovule morphology

Flower buds were collected from W78-122 at stages from immature to anthesis. Flowers were dissected, size of the ovary was measured, and number of viable-appearing ovules were counted. Ovules from immature and mature ovaries were collected and cleared for direct observation. Ovules were fixed in FPA₅₀ (40%formaldehyde:propionic acid:50%ethanol, 5:5:90, v:v:v) for 24 hr (Herr, 1974a). Ovules were cleared using Crane's (1978) technique. Fixed ovules were dehydrated in a 50-70-85-100-100% ethanol series for 30 min each. They were then brought to 100% methyl salicylate through 2 changes for 30 min each in a 1:1 and 1:3 ethanol:methyl salicylate mixture. Finally they were transferred to 100% methyl salicylate overnight, and mounted on a Raj slide (Herr, 1974b) in the same solution. Observation of the cleared ovules was done with a Nikon Normarski differential interference contrast microscope at a magnification of 400x.

Phenotype of F₁

Cross-pollinations of W78-122 (pollen parent) with 4 different V. ashei cultivars were conducted during the spring of 1980 by Dr. P. M. Lyrene. In the spring of 1981, seedlings of the populations were planted into the high-density nursery with seedlings from the breeding program. Stem diameter, height, and internode length of all F₁ plants and plants from 3 unrelated populations were measured in February 1983. Ten leaves from: all F₁ plants, plants from 3 populations which did not have W78-122 in the pedigree, ramets of 2 V. ashei cultivars, and 3 ramets of W78-122 were collected and leaf area was measured as above. All plants were growing in a high-density nursery.

Female fertility of the F_1 plants was monitored for 3 years by recording the plants which flowered and fruited. Plants which produced flowers but never had fruit were considered female-sterile.

Results and Discussions

Phenotype in its native environment

Height, trunk diameter, and internode length were larger in W78-122 than in most of the other measured plants growing in the same vicinity (Table 5-1). Leaf area was more than 2.5X greater in W78-122 than on the average check plant (Table 5-2). In a normal population with mean and variance like that of the 10 sampled "check plants", only 1 plant in 100,000,000 would be expected to have a leaf area as great as that of W78-122. Leaf index, a measure of leaf shape, was not significantly different among W78-122 and the check plants but was highly variable (Table 5-2). The undersurface of the leaves of W78-122 was totally void of stalked glands. While this is considered by some to be a characteristic of V. ashei, 2 of the other native rabbiteye plants sampled also lacked stalked glands on their leaf undersurface (Table 5-2). Fruit has never been observed on W78-122 during 7 years despite abundant flowering each year (Lyrene, per. comm).

Phenotype at the Horticultural Research Unit

A ramet of W78-122 growing at the HRU had a larger leaf area than any of the native or cultivated V. ashei plants measured (Table 5-3). This ramet has flowered for 4 years without setting fruit. Superficially, the flowers appear normal, although rather small for V.

Table 5-1. Height, trunk diameter and internode length of the 10 largest V. ashei plants growing with W78-122 in an abandoned blueberry plantation near Gainesville, Florida.

Plant	Height (m)	Trunk diameter ^z (mm)				Internode length (cm)
		1	2	3	Mean	
W78-122	6.0	56	55	51	54 ^a ^y	2.6
Check 1	5.5	43	42	39	41bc	2.0
2	4.0	42	38	38	39c	2.3
3	3.8	35	28	27	30de	2.5
4	3.1	32	32	21	28de	2.5
5	3.5	32	29	29	30de	1.9
6	3.9	35	30	30	32d	1.9
7	5.0	55	45	37	46b	1.7
8	4.5	48	45	43	45bc	2.6
9	3.4	25	25	24	25e	1.6
10	4.5	50	42	42	45bc	2.3

^z Average diameter of the 3 largest trunks at a height of 1.5 m above ground.

^y Mean separation within columns by Duncan's Multiple Range Test, 5% level.

Table 5-2. Leaf area and leaf index comparisons of 20 *V. ashei* plants with W78-122 growing in an abandoned blueberry plantation near Gainesville, Florida.

Plant	Leaf area ^z (cm ²)		Leaf index ^y	
	Mean	SD	Mean	SD
W78-122				
Rep 1#	37.0	3.3	1.8	.12
Rep 2#	36.4	7.2	1.8	.08
Check 1	12.2	1.7	1.8	.15
2	14.3	2.3	2.0	.14
3	17.0	2.3	1.8	.10
4	14.4	2.4	1.7	.10
5	10.4	1.8	2.1	.30
6	11.3	1.3	1.8	.10
7	13.3	2.0	2.1	.10
8	12.7	2.0	1.8	.10
9	11.8	1.4	2.1	.10
10	14.6	1.1	1.7	.05
11	19.7	2.6	1.7	.20
12	13.0	1.8	1.8	.10
13	11.5	2.0	2.4	.20
14	24.2	4.1	1.7	.10
15	12.6	1.3	1.6	.10
16	11.3	1.4	1.8	.10
17	13.2	1.5	1.6	.20
18#	15.1	1.9	1.8	.10
19	13.3	2.3	2.0	.20
20#	16.5	2.7	2.2	.20
W78-122 (Combined)	36.7 a ^x	5.5	1.8 a ^x	.10
Checks (Combined)	14.1 b	3.3	1.9 a	.21

^z Surface area measured using a LiCor Portable Surface Area Meter Model 3000.

^y Length/width.

^x Mean separation within columns using Duncan's Multiple Range Test, 5% level.

^w Mean of 10 leaves measured on each plant. Two replications of 10 leaves were collected from W78-122.

#No stalked glands on the undersurface of leaves.

Table 5-3. Leaf area comparisons of native and cultivated *V. ashei* with W78-122 growing at the Horticulture Research Unit.

Clone	Type	Leaf area (cm ²)	
		Mean	SD
W78-122	Native	25.6a ^z	5.3
W78-34	"	6.0de	.8
W78-66	"	5.3e	1.4
W78-35	"	11.8c	2.6
W78-74	"	8.2d	1.4
W78-37	"	6.8de	1.2
W78-39	"	8.4d	1.4
Bluebelle	Cultivar	10.7c	1.7
Climax	"	10.6c	3.0
Fla K	"	16.9b	2.2
Beckyblue	"	12.0c	3.0

^zMean separation within columns using Duncan's Multiple Range test, 5% level.

ashei, with normal-appearing calyx, corolla, pistil, and anthers. Anthesis appears to be normal. Flowering superficially appears to be proceeding normally until a week or two after anthesis, when the flowers abscise. Flower abscission occurs at about the same time in the reproductive cycle as the abscission of unpollinated flowers on normal plants.

Chromosome counts

Approximately 36 bivalent chromosomes were counted in 32 pollen mother cells undergoing meiosis. This indicated that W78-122 is most likely a member of the hexaploid species V. ashei with a chromosome count of $2n=6x=72$.

Male fertility

Male fertility, measured as percent pollen germination on an artificial medium and fruit set of unrelated V. ashei cultivars pollinated with W78-122, was normal when compared with 19 other native V. ashei clones tested from north Florida and south Georgia (Table 5-4). Pollen germination of W78-122 on the sucrose medium was similar to pollen germination of 7 other native plants and much greater than 13 plants. The variability in percent pollen germination of W78-122 was large, but it was also large for some of the other clones. Percent fruit set when pollen from W78-122 was used to pollinate V. ashei cultivars was higher than that of 5 other clones, less than 3, and similar to the others. Male fertility in V. ashei appears to be similar to that found in V. angustifolium (Hall and Aalders, 1961).

Table 5-4. Male fertility compared for W78-122 and 19 other native clones of V. ashei from south Georgia and north Florida measured by pollen germination on an artificial medium and fruit set after pollination of known fertile plants.

Clone	Pollen germination ^z (%)		Fruit set when pollen put onto <u>V. ashei</u> cultivars ^y (%)
	Mean	Range	
W78-122	67	43-84	62
W78-72	73	53-90	89
W78-112	22	13-29	72
W78-77	22	14-29	56
W78-74	39	34-50	38
W78-39	73	54-77	66
83-42	08	05-14	53
83-44	41	27-52	67
83-40	40	26-53	61
83-43	43	15-76	56
W78-102	33	21-43	77
W78-34	43	20-65	53
83-47	59	52-68	57
W78-41	36	25-52	29
W78-40	35	16-57	38
83-46	57	40-77	37
83-45	33	27-39	58
83-41	66	30-85	76
W78-69	62	52-75	33
W78-66	23	10-39	63

^z10% sucrose - .01% boron solution for 4 hours, 5 samples per clone.

^yNumber of fruit/pollination on V. ashei cultivars when the native plants are the pollen parents. About 30 flowers pollinated for each test.

Pollen germination and growth in pistil

Pollen had germinated on the stigma W78-122 and 7 other native V. ashei plants within 12 hours (Table 5-5). While pollen tube growth through the style and into the ovary was highly variable among the 8 clones, pollen tube growth of W78-122 was similar to pollen tube growth of W78-112. Long intervals between sampling times may accentuate differences in reported pollen tube growth rates. Sterility in this plant is therefore probably not due to abnormalities of the stigma and style which would block the pollen tube growth in the style and into the ovary.

Ovary and ovule morphology

Ovule degeneration occurred during the development of the flower (Table 5-6). Dissected ovaries from young flowers of W78-122 contained ovules with the normal number and appearance (Figure 5-1). Dissected ovaries from flowers at anthesis contained a few, white, plump healthy looking ovules, but the majority were abnormal. Some abnormal ovules were white but misshapen, some clear and thin, and some very small and white. Cleared ovules from flowers at anthesis had not completely bent over and were not anatropous. This seemed to indicate a cessation of or delay in development. One abnormal ovule contained an embryo sac in which only 2 nuclei could be seen instead of the mature megagametophyte expected (Stushnoff and Palser, 1970).

It appears that female sterility in W78-122 is due to failure of the megagametophyte to develop normally. Meiosis was studied only in the microspore mother cell and was not attempted in the megaspore mother cell. Since normal meiosis was observed in the microsporocyte, abnormal

Table 5-5. Number of hours from pollination to pollen germination and growth in cross-pollinated pistils of W78-122 and 7 other native V. ashei plants.

Clone	Pollen germination on stigma	Pollen tubes midway in style	Pollen tubes in ovary
83-42	12 hr	36 hr	48 hr
W78-39	12	24	72
W78-40	12	36	48
W78-66	12	48	72
W78-72	12	48	>96
W78-77	12	48	>96
W78-112	12	72	>96
W78-122	12	72	96

Table 5-6. Ovule number and appearance of immature and mature flowers of W78-122 and the V. ashei cultivar 'Bluebelle'.

Flower	Immature flowers ^z		Mature flowers ^y			
	W78-122 ovules		W78-122 ovules		Bluebelle ovules	
	(no.)		(no.)		(no.)	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
1	50-60	0	13	11	53	0
2	"	"	1	20	47	"
3	"	"	3	10	51	"
4	"	"	3	13	49	"
5	"	"	7	9	55	"
6	"	"	5	7	58	"
7	"	"	6	11	46	"
8	"	"	8	3	47	"
9	"	"	5	20	54	"
10	"	"	17	11	50	"

^zFlowers from newly expanded inflorescence bud, less than 3 mm long.

^yFlowers at anthesis.



Figure 5-1. Dissected ovaries of W78-122 (bottom) and *V. ashei* cultivar 'Bluebelle' (top) from flowers at anthesis.

meiosis in the megasporocyte, if it is present in W78-122, is probably not due to wide hybridization or chromosomal abnormalities.

Phenotype of F_1

Stem diameter, height, and internode length were not greatly different in seedlings produced by crossing W78-122 with V. ashei cultivars compared with comparable seedling populations from V. ashei crosses not involving W78-122 (Table 5-7). Populations with W78-122 as the pollen parent averaged slightly higher in stem diameter, height, and internode length compared to the other populations, but there was much variability.

Female sterility segregated in 3 of the W78-122 populations (Table 5-8). All plants within the 'Bluebelle' x W78-122 population which flowered also bore some fruit. All plants of this population flowered at least 1 of the 3 years that measurements were taken. In the other 3 populations, female sterility ranged from 11% to 25%. The percent of female-sterile plants in these 3 population decreased each year. The last year that measurements were taken the plants were 6 years old. Three plants in 1 population and 1 plant in each of the other 2 populations did not flower any of the measured years. This lack of flowering may have been due to the close spacing of plants in the test nursery.

Leaf area of 3 of the F_1 populations with W78-122, was larger than with the check populations or the cultivar ramets (Table 5-9). The 'Bluebelle' x W78-122 population was not statistically different than 1 of the check populations. In the 'Bluebelle' x W78-122 and 'Climax' x W78-122 populations, leaf area was less than the mid-parent value.

Table 5-7. Shoot diameter, height, and internode length of seedlings from V. ashei cultivars crossed with W78-122 compared to seedlings from V. ashei crosses not involving W78-122.

Population	Plants (no.)	Stem diameter (mm)		Height (cm)		Internode length (cm)	
		Mean	Range	Mean	Range	Mean	Range
Climax x W78-122	19	13.9 ^a ^z	4.5-28.0	154 ^a ^z	54-200	1.5 ^a ^z	.9-2.1
Beckyblue X W78-122	34	11.9 ^{ab}	6.0-18.0	146 ^a	58-224	1.5 ^a	1.0-2.4
Fla K X W78-122	18	14.1 ^a	9.0-18.5	148 ^a	91-180	1.4 ^a	1.0-1.8
Bluebelle X W78-122	19	16.1 ^a	12.0-18.5	164 ^a	124-196	1.3 ^a	.9-1.6
Climax X Comp. 1 ^y	20	12.9 ^a	4.5-20.0	148 ^a	45-180	1.4 ^a	1.0-2.8
Bluebelle X Comp. 2	12	12.1 ^a	5.5-22.0	145 ^a	54-172	1.2 ^a	.9-1.6
Beckyblue X Comp. 3	20	10.6 ^b	6.0-18.0	114 ^b	84-166	1.2 ^a	.7-1.8

^zMean separation within columns using Duncan's Multiple Range Test, 5% level.

^yComposite 1,2: pollen from 10 or more V. ashei advanced selections from the breeding program. Composite 3: pollen from 5 late-ripening native V. ashei plants.

Table 5-8. Segregation of female sterility and fertility in F-1 populations of female fertile V. ashei cultivars x W78-122.

Population	Seedlings evaluated (no.)	Female fertile (no.)	Female sterile (no.)	Female fertility unknown (no.)
Beckyblue X W78-122	34	27	4	3
Climax X W78-122	20	11	5	4
Bluebelle X W78-122	19	19	0	0
Fla K X W78-122	18	14	2	2

²For reasons see text.

Table 5-9. Leaf area of V. ashei populations with and without W78-122 parentage.

Population	Plants (no.) ^z	Leaf area (cm ²)	
		Mean	Range
W78-122 (Ramets)	3	25.7 a ^y	17.0-39.6
Climax (Ramets)	4	9.1 f	7.3-11.6
Bluebelle (Ramets)	2	10.6 ef	7.5-13.4
Bluebelle x W78-122	19	13.1 cd	7.1-18.4
Beckyblue x W78-122	25	14.3 bc	7.1-22.2
Climax x W78-122	17	15.4 b	7.0-25.6
Fla K x W78-122	18	14.3 bc	8.1-27.0
Beckyblue X Comp 1 ^x	7	9.2 f	4.6-17.1
Climax X Comp 2 ^x	18	11.9 de	7.1-22.0
Bluebelle X Comp 3 ^x	7	10.6 ef	6.6-14.8

^zTen leaves were averaged for each plant.

^yMean separation within columns by Duncan's Multiple Range Test 5% level.

^xComp. 1 and 2= composite of pollen from approximately 10 advanced V. ashei breeding clones. Comp. 3= composite of pollen from 5 late-ripening native V. ashei clones selected from the woods in Alachua County, FL.

Ramets of the clones Fla K and 'Beckyblue' were not growing in the high density areas and midparent comparisons could not be made. Female-sterility and large leaf size did not appear to be correlated within the F_1 populations (Table 5-10). The F_1 plants were considered fertile if any fruit were set. It is possible that female fertility in some of the plants may be impaired. Also, large leaf size may only be expressed in mature plants and it may be too early to see differences.

Patterns of inheritance are not known in V. *ashel*. It is not known whether most loci exhibit polyploid or diploid inheritance. The lack of female sterility in the 'Bluebelle' x W78-122 population and its presence in the other 3 populations may indicate the segregation of other characteristics affecting female fertility. 'Bluebelle' is known as a cultivar that consistently sets fruit well in Florida, and modifier genes from 'Bluebelle' may be suppressing the action of the female-sterile trait in the F_1 hybrids. It is also possible that the F_1 plants classified as sterile in the other crosses after 3 years flowering could set some fruit in later years. Backcross and F_2 populations were made, but fertility can not be assessed in these populations until spring 1986.

Table 5-10. Leaf area and female-fertility of F-1 populations from crosses between cultivated V. ashei clones and W78-122.

Population	Seedlings (no.)	Leaf area (cm)	
		Mean ^z	SD
Beckyblue X W78-122	34	14.3a	2.11
Fertile ^y	27	14.3a	2.10
Sterile ^y	4	14.1a	2.37
Did not flower ^x	2	—	—
Undetermined ^w	1	—	—
Fla K X W78-122	18	14.3a	2.64
Fertile	14	14.1a	2.37
Sterile	2	16.7a	4.67
Did not flower	2	—	—
Climax X W78-122	20	15.4a	3.54
Fertile	11	15.2a	3.80
Sterile	5	15.9a	3.08
Did not flower	1	—	—
Undetermined	3	—	—
Bluebelle X W78-122	19	13.1	1.92
Fertile	19	—	—
Sterile	0	—	—
Did not flower	0	—	—

^zBased on 10 fully expanded leaf per plant.

^yFlowered at least once during the 3 years 1983, 1984, 1985 but produced no berries during any of the 3 years.

^xDuring years when plants were 3, 4, and 5 years old.

^wFlowered the first year (1983) but either died or did not flower during 1984 or 1985.

^uMean separation within groups by Duncan's Multiple Range Test, 5% level.

SECTION VI

SUMMARY AND CONCLUSIONS

Expression and Inheritance of Dwarfism in *Vaccinium ashei* (Reade)

Vaccinium ashei W78-66 is a short-internode, compact plant with small, dark-green, semievergreen leaves and small black fruit. It is hexaploid, as are the other members of the species, and fertile gametes are produced following predominantly normal meiosis. The short-internode trait is due predominantly to fewer pith parenchyma cells per internode and, to a lesser degree, smaller pith parenchyma cells. This dwarf is sensitive to exogenously-applied GA_3 . Internode lengths equal in size to nondwarfs result from applications of GA_3 at 500 mg/liter or greater. Number of nodes and length of new shoots also increase with the above treatment.

Vaccinium ashei W78-66 grew as well in vitro as the nondwarf on modified ARM containing 5 mg/liter 2IP. Colonies produced by the dwarf were shorter and had shorter internodes than nondwarf colonies, but were equally prolific in shoot production. The dwarf was sensitive to GA_3 amended medium; height and shoot number per vial increased with increasing levels of GA_3 . Shoot number per vial also increased with increasing GA_3 level in the nondwarf. The dwarf and the nondwarf responded similarly to high and low levels of the cytokinin 2IP. High levels resulted in the death of many plants and reduced height, shoot number, and colony diameter. The nondwarf appears slightly more tolerant to high cytokinin than the dwarf.

Vaccinium ashei W78-66 crosses readily with other members of the same species. The short-internode trait can be selected in the F_1 population from the cross W78-66 x tall V. ashei cultivars. This short-internode trait is also expressed in some of the progeny when selected F_1 plants are backcrossed to tall cultivars.

The fact that a short-internode phenotype was evidenced by at least some F_1 plants from crosses between W78-66 x tall cultivars has important implications in a breeding program. Dwarfing has become increasingly important in the development of modern fruit cultivars. Because this trait appears to be easy to move into improved cultivars, high-quality dwarf cultivars could possibly be developed after only 2 or 3 backcross generations.

Self-incompatibility in *Vaccinium ashei* (Reade)

Nineteen native clones of *Vaccinium ashei* varied in relative self-fruitfulness from 0.0 to 68%. Relative self-fertility varied from 0% to 110% when measured as the number of seeds per fruit when selfed divided by the number of seeds per fruit when crossed. When measured as the relative number of seedlings per pollination it varied from 1% to 45%. All clones were male fertile and all but 1 clone were female fertile. Compatible and incompatible pollen tubes grew the full length of the style in approximately the same time span. The level of self-fruitfulness was insufficient for the successful use of solid block plantings of these clones.

Female Sterility in *Vaccinium ashei* (Reade)

The large-leaved, female-sterile, male-fertile blueberry W78-122 is hexaploid and most likely a variant form in the species V. ashei. Female sterility appears to be due to the abnormal development of the megagametophyte or ovule rather than to wide hybridization or chromosome rearrangements. Male fertility is normal. Three F_1 populations segregated for female fertility while 1 population was completely fertile.

APPENDIX

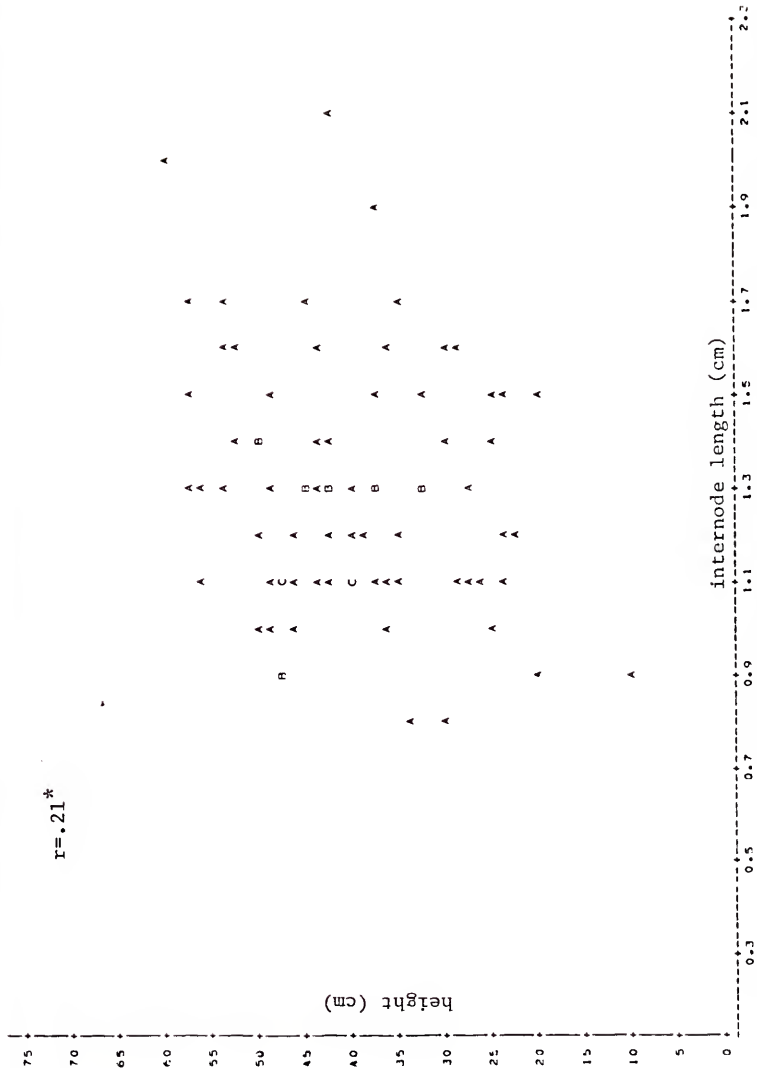


Figure 1. Plot of height by internode length in the population 78-41 x 83-4 (W78-66 not in the pedigree).

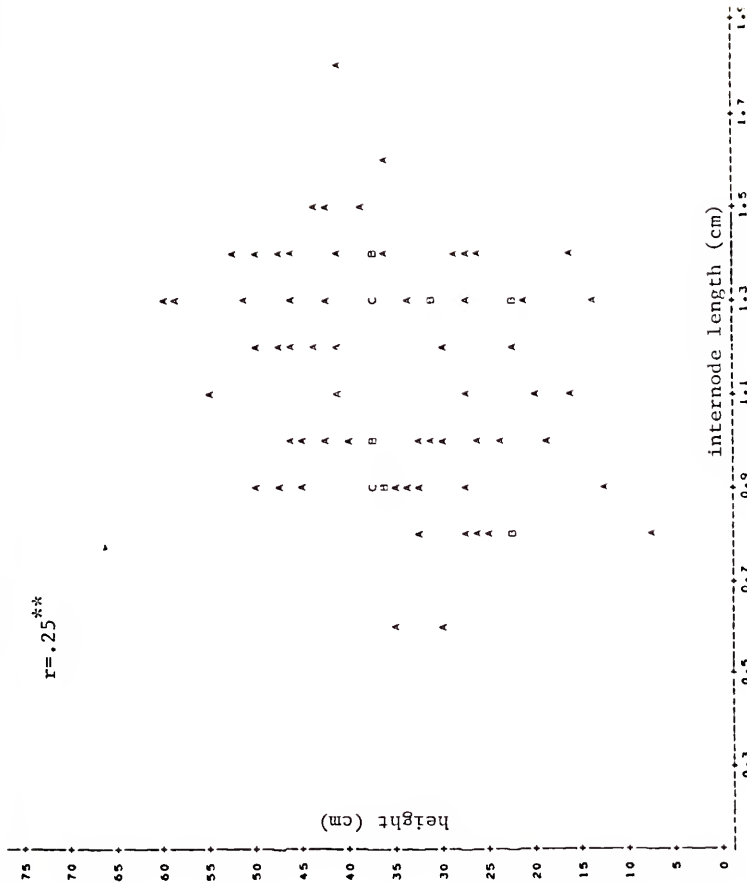


Figure 2. Plot of height by internode length in the population 78-69 x 83-17 (W78-66 not in the pedigree).

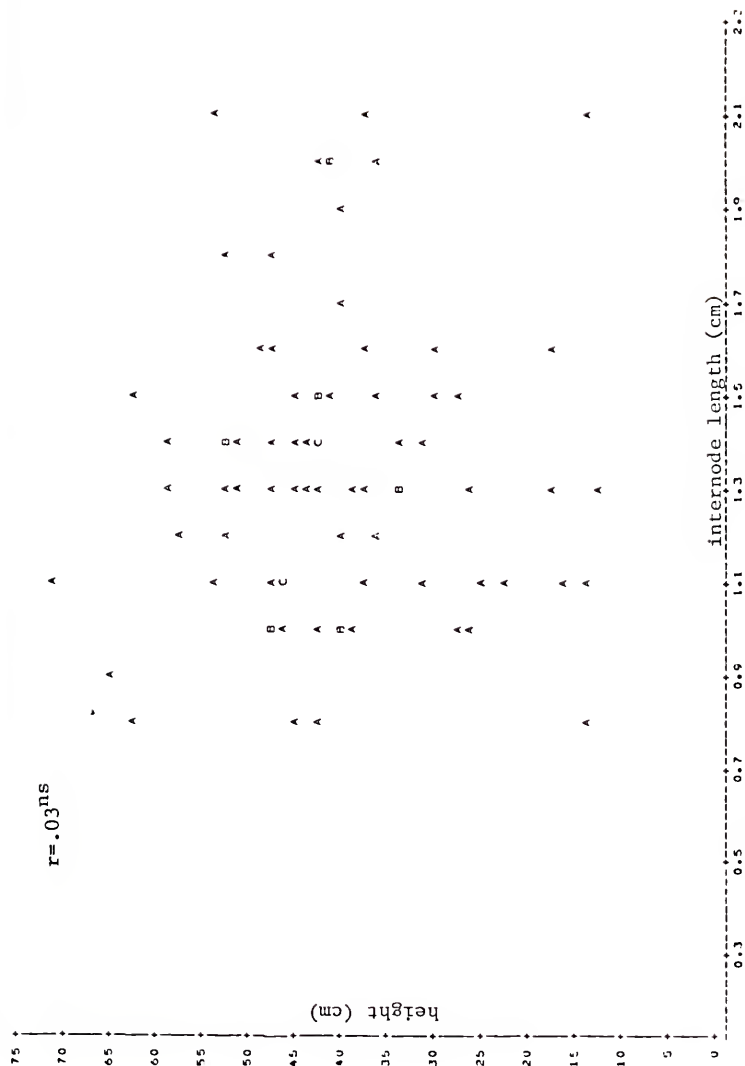


Figure 3. Plot of height by internode length in the population 83-44 x 83-24 (w78-66 not in the pedigree).

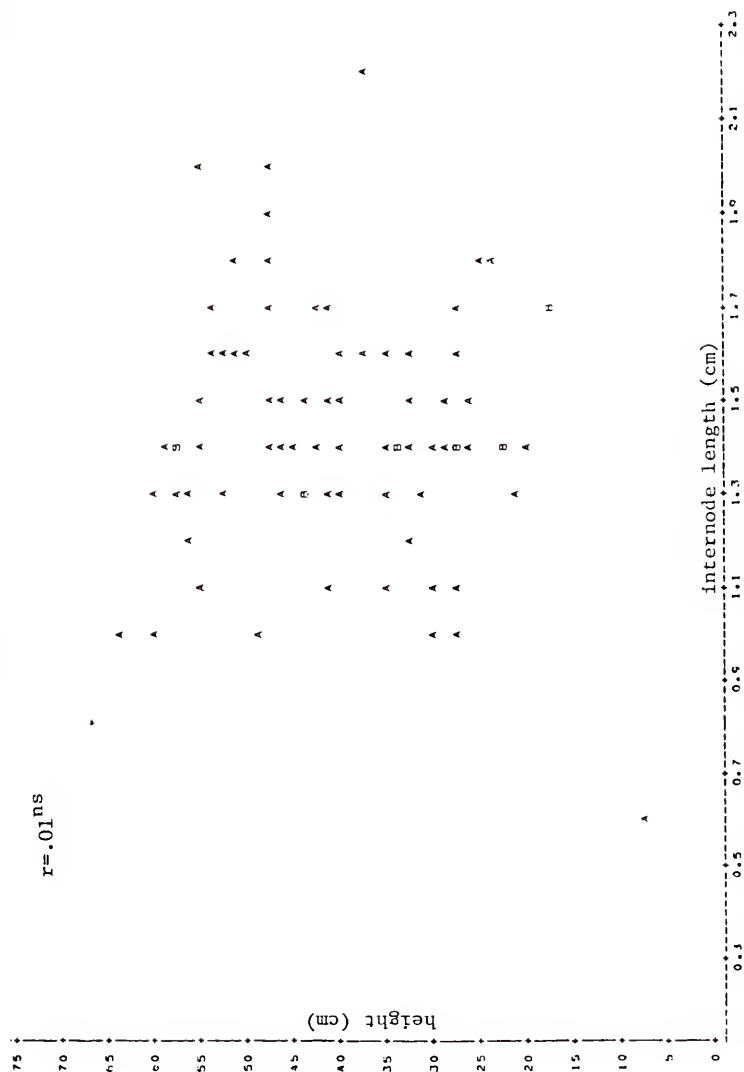


Figure 4. Plot of height by internode length in the population 83-45 x 83-19 (W78-66 not in the pedigree).

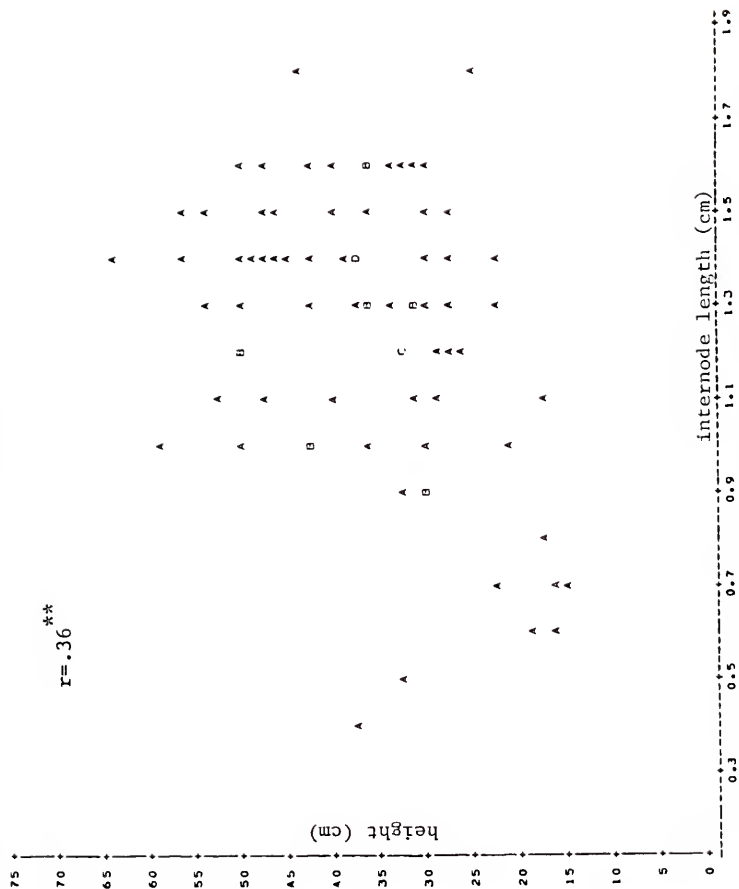


Figure 5. Plot of height by internode length in the population 'Aliceblue' x 82-167 [tall cultivar x (W78-66 x tall cultivar)], where the F_1 parent was selected for short internodes.

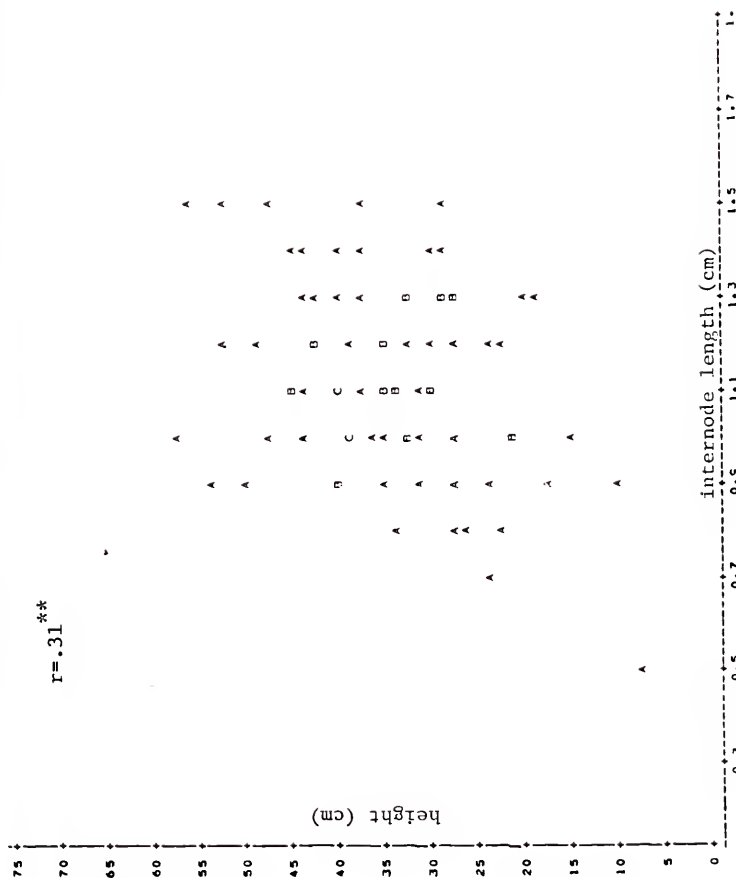


Figure 6. Plot of height by internode length in the population 'Bonita' x 82-201 [tall cultivar x (W78-66 x tall cultivar)], where the F_1 parent was selected for short internodes.

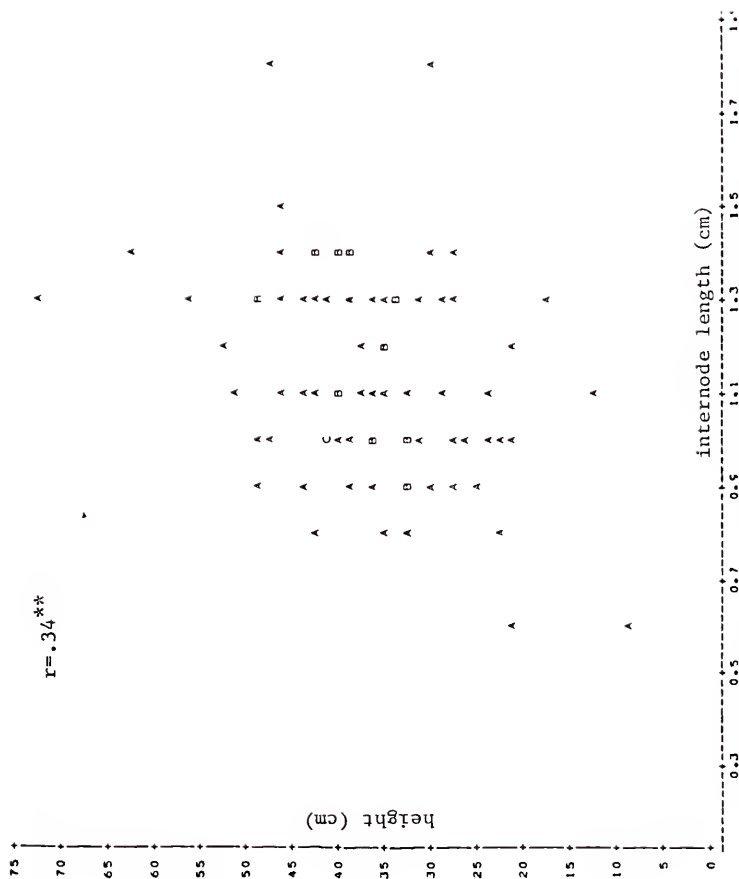


Figure 7. Plot of height by internode length in the population 'Bluebelle' x 82-166 [tall cultivar x (W78-66 x tall cultivar)], where the F_1 parent was selected for short internodes.



Figure 8. Plot of height by internode length in the population 'Bluebelle' x W78-66 (tall cultivar x W78-66).

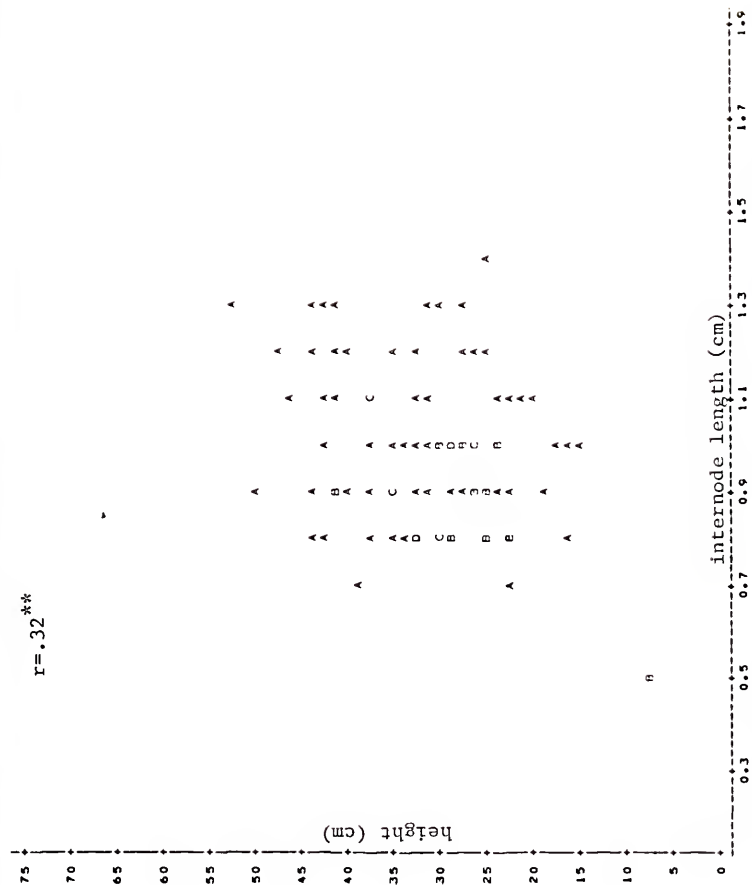


Figure 9. Plot of height by internode length in the population 82-201 x 82-197 [(W78-66 x tall cultivar) x (W78-66 x tall cultivar)], where both parental F₁'s were selected for short internodes.

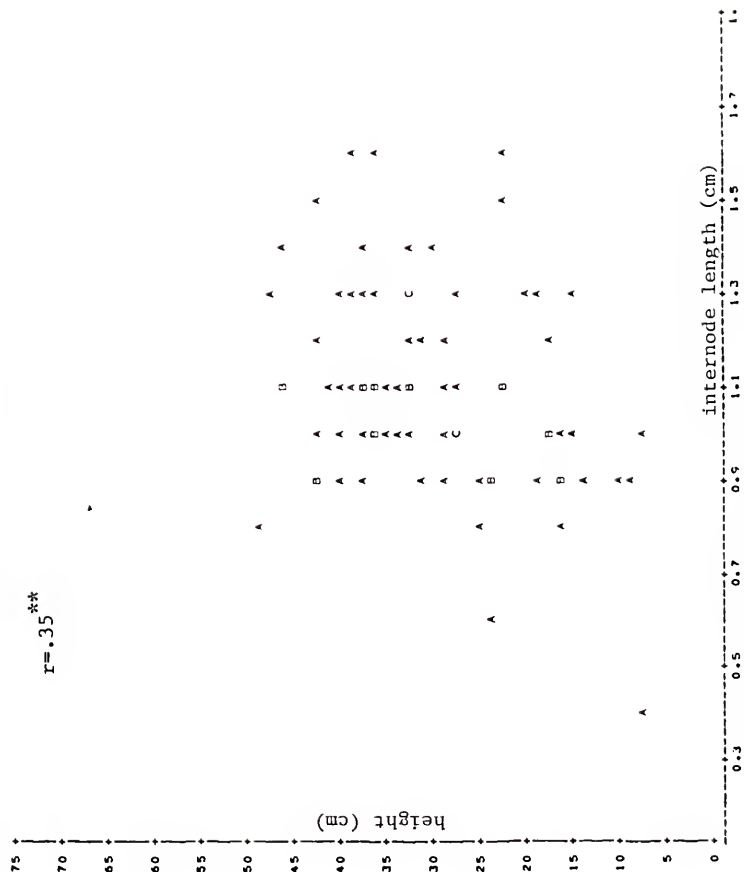


Figure 10. Plot of height by internode length in the population 82-158 x 82-160 [(w78-66 x tall cultivar) x (w78-66 x tall cultivar)]₁, where both parental F₁'s were selected for short internodes.

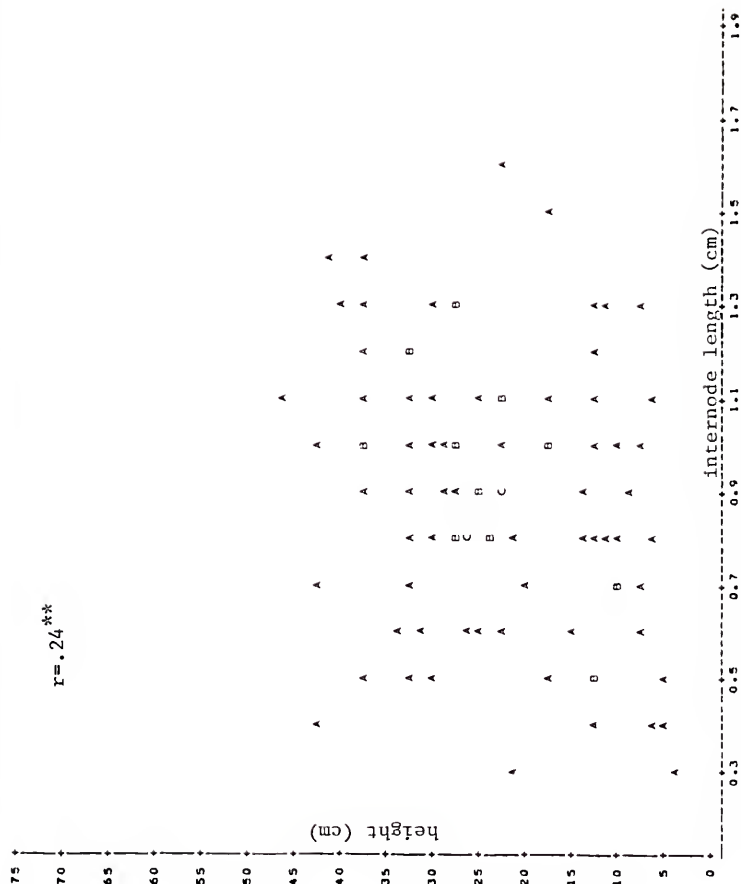


Figure 11. Plot of height by internode length in the population 82-160 x 82-161 [(W78-66 x tall cultivar) x (W78-66 x tall cultivar)], where both parental F_1 's were selected for short internodes.

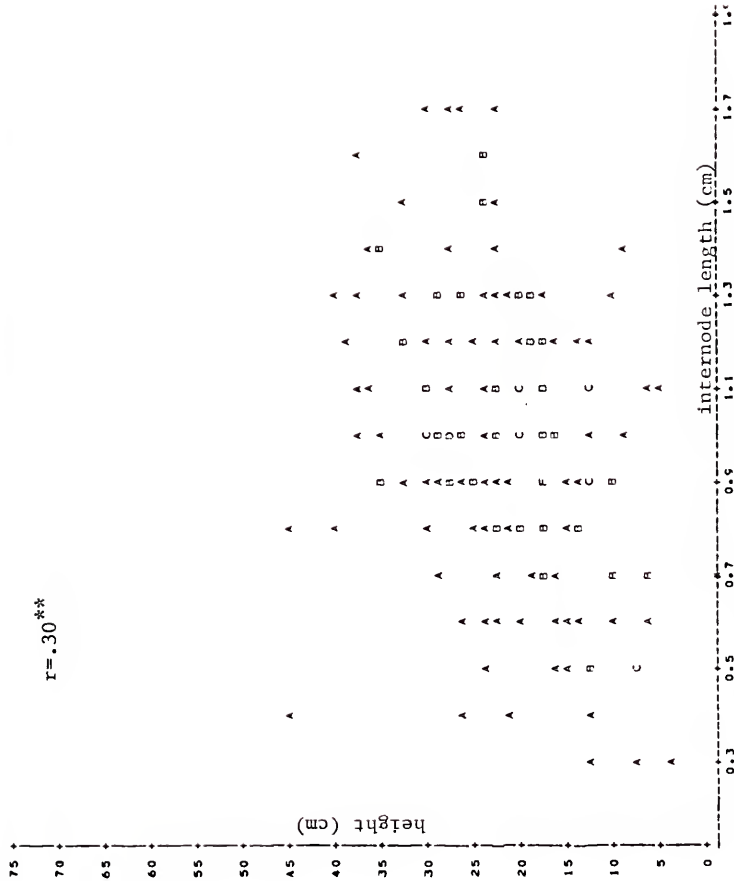


Figure 12. Plot of height by internode length in the population 82-161 x 82-156 [(W78-66 x tall cultivar) x (W78-66 x tall cultivar)], where both parental F_1 's were selected for short internodes.

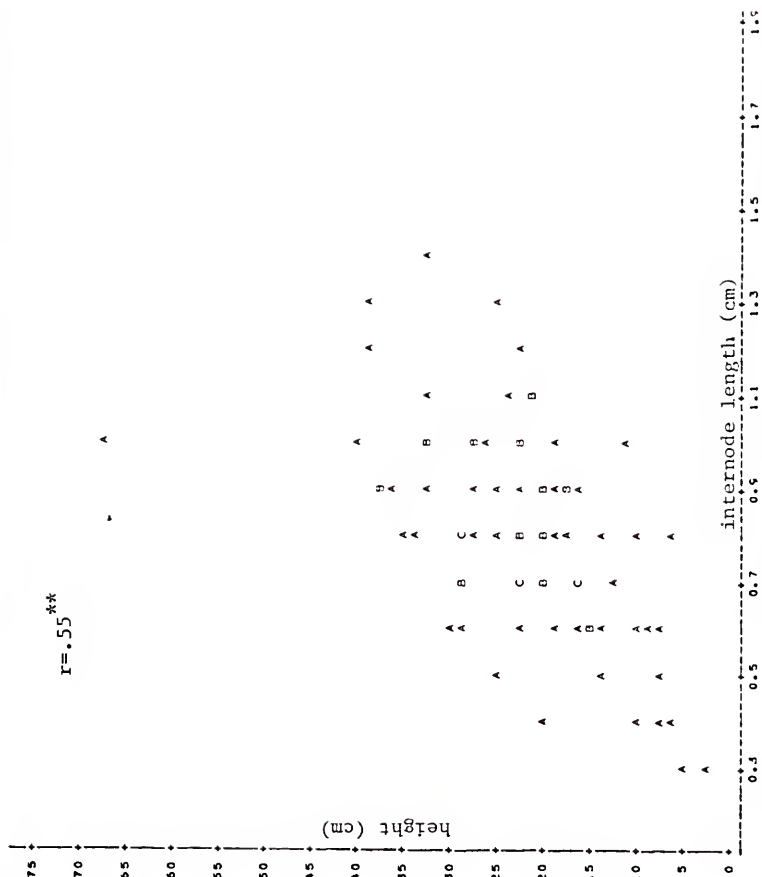


Figure 13. Plot of height by internode length in the population 82-195 x W78-66 [(W78-66 x tall cultivar) x W78-66], where the parental F_1 was selected for short internodes.

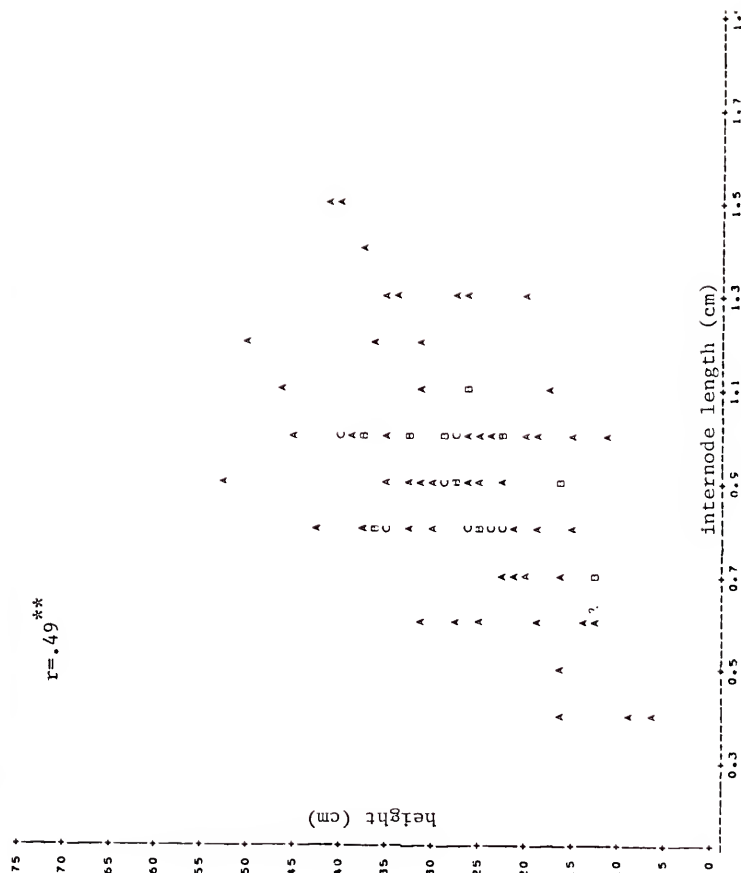


Figure 14. Plot of height by internode length in the population 82-196 x W78-66 [(W78-66 x tall cultivar) x W78-66], where the parental F_1 was selected for short internodes.

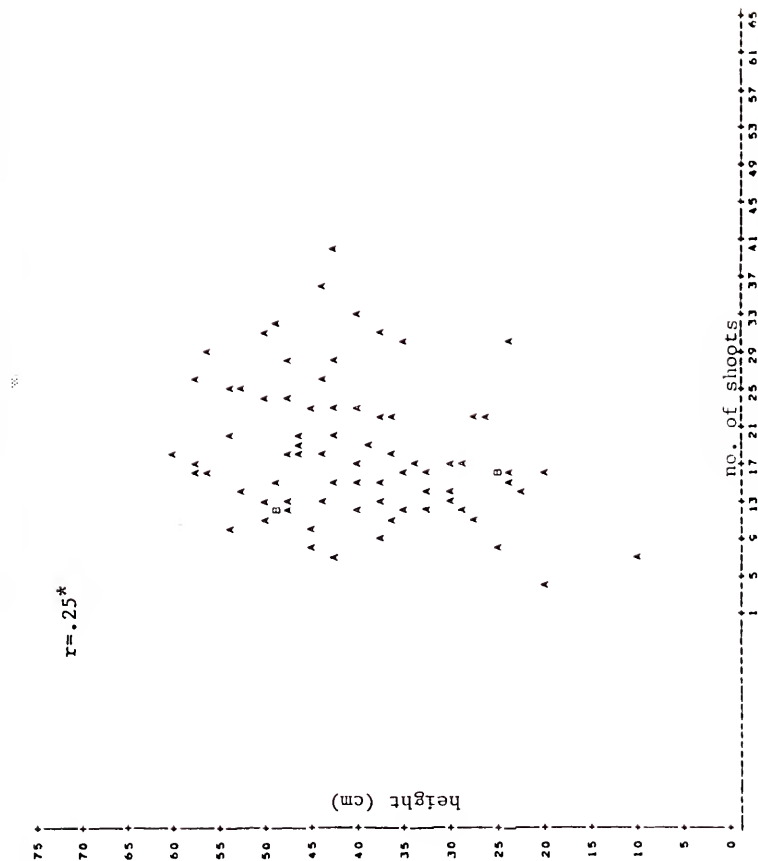


Figure 15. Plot of height by number of shoots in the population 78-41 x 83-4 (w78-66 not in the pedigree).

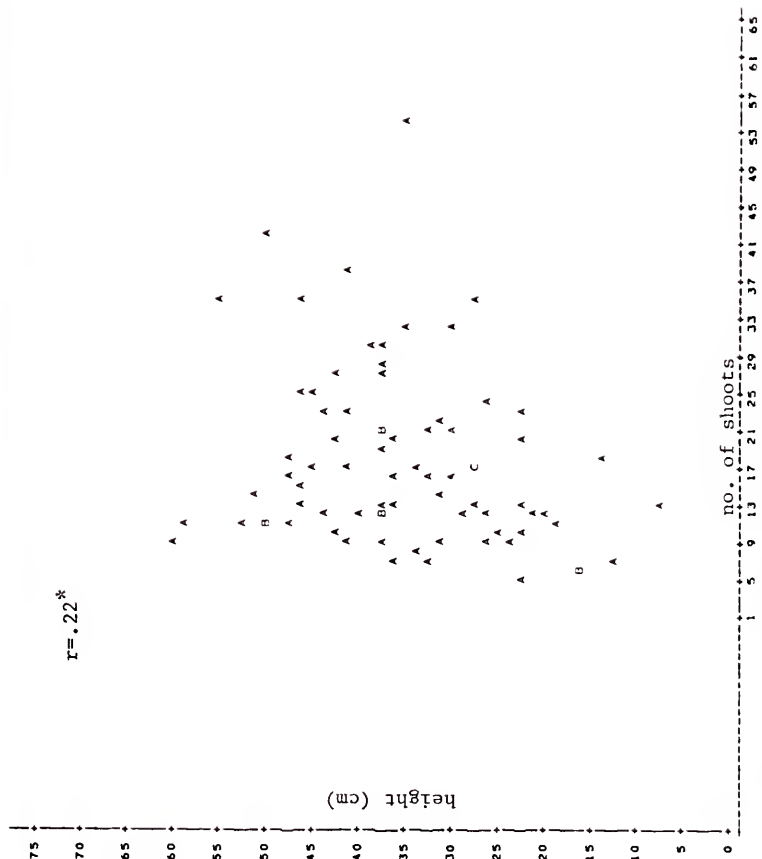


Figure 16. Plot of height by number of shoots in the population 78-69 x 83-17 (W78-66 not in the pedigree).

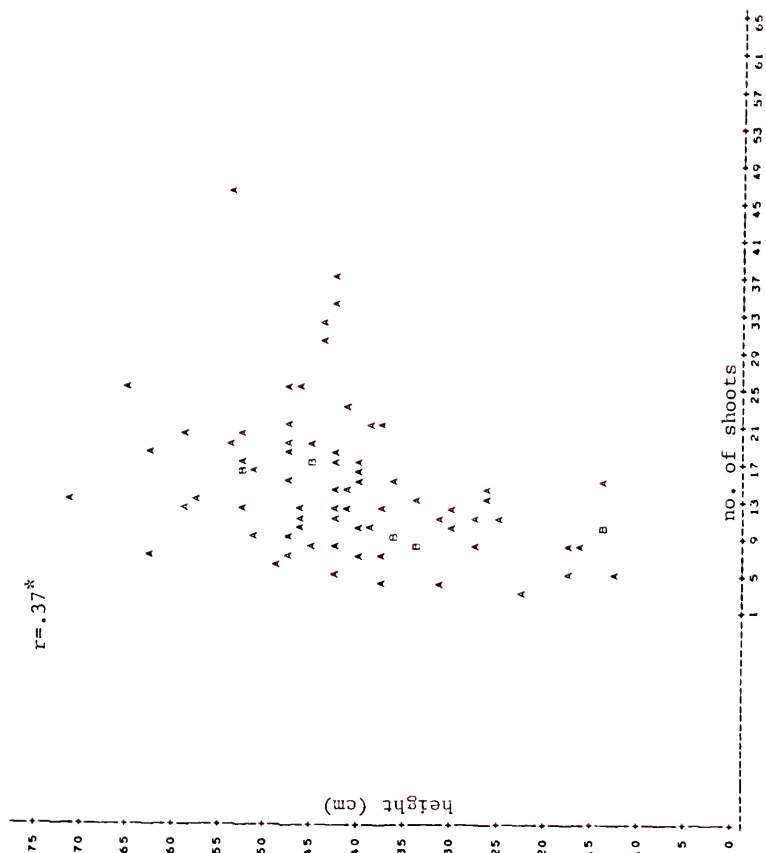
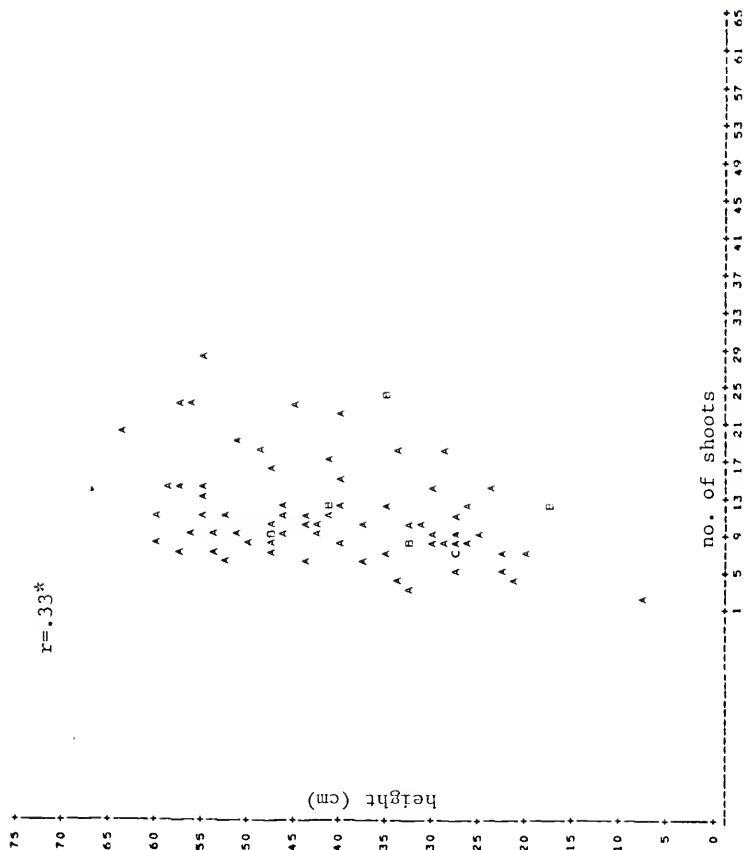


Figure 17. Plot of height by number of shoots in the population 83-44 x 83-24 (W78-66 not in the pedigree).



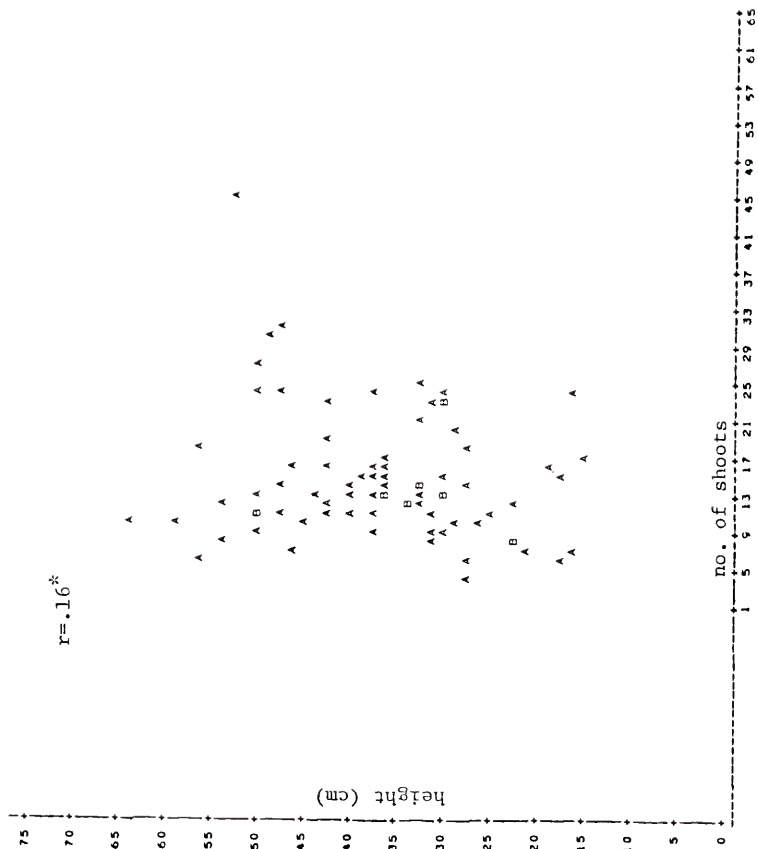


Figure 19. Plot of height by number of shoots in the population 'Aliceblue' x 82-167 [tall cultivar x (W78-66 x tall cultivar)], where the F_1 was selected for short internodes.

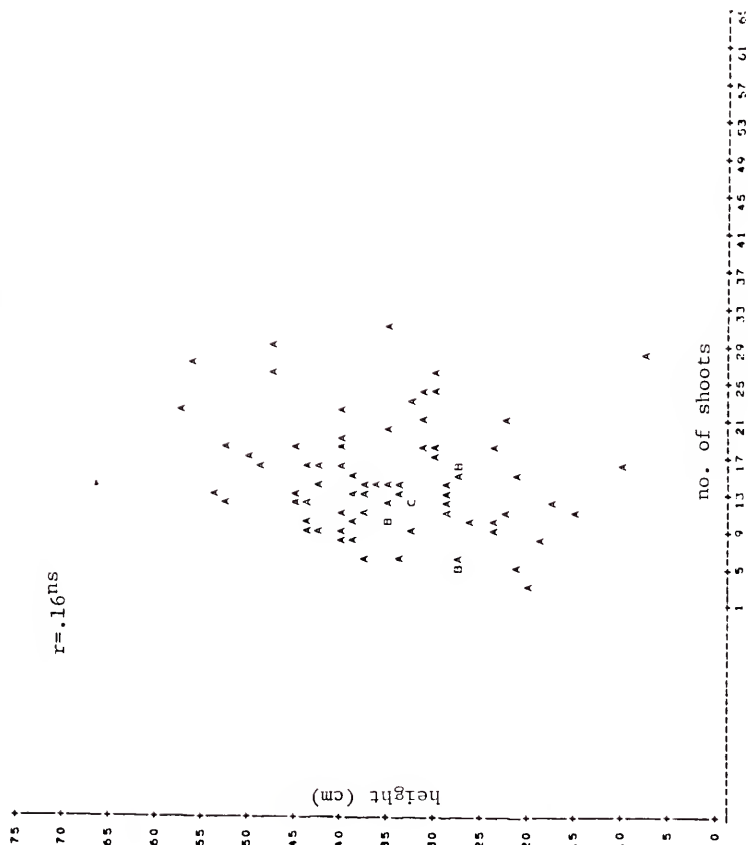


Figure 20. Plot of height by number of shoots in the population 'Bonita' x 82-201 [tall cultivar x (W78-66 x tall cultivar)], where the F_1 was selected for short internodes.

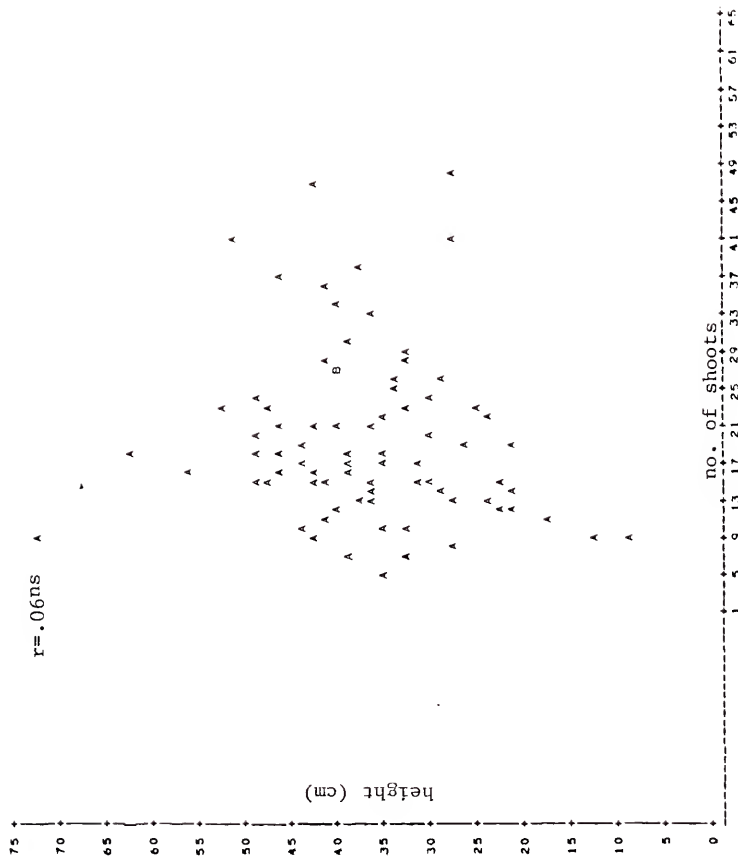
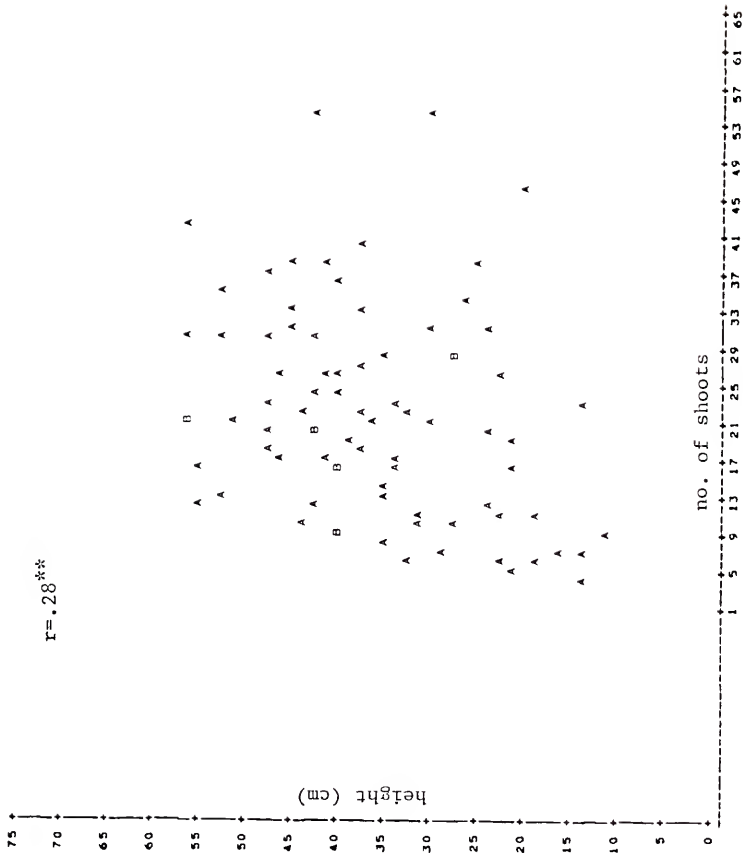


Figure 21. Plot of height by number of shoots in the population 'Bluebelle' x 82-166 [tall cultivar x (w78-66 x tall cultivar)], where the F_1 was selected for short internodes.



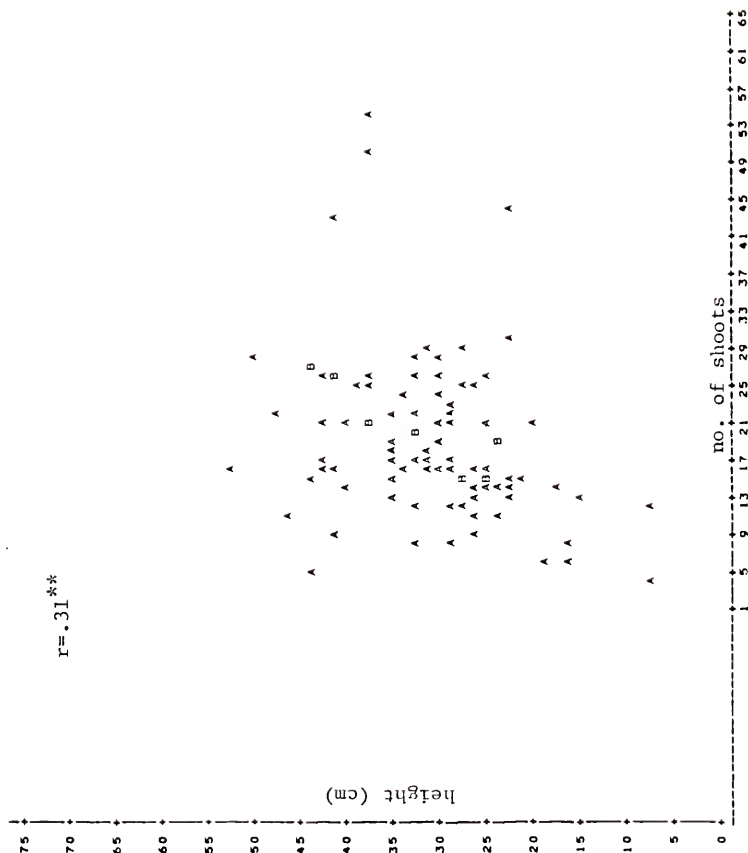


Figure 23. Plot of height by shoot number in the population 82-201 x 82-197 [(W78-66 x tall cultivar) x (W78-66 x tall cultivar)], where both parental F₁'s were selected for short internodes.

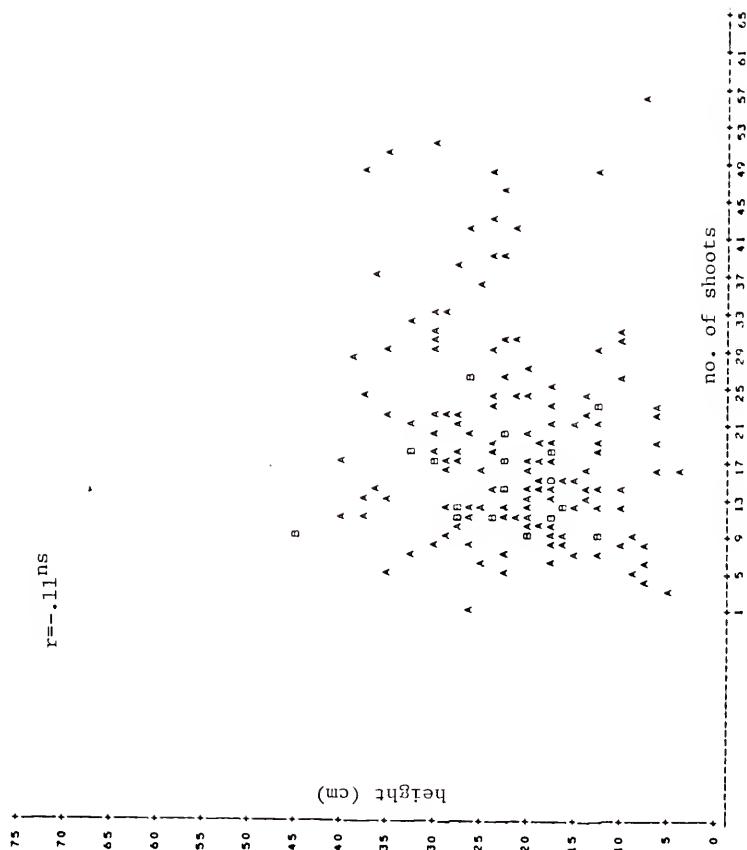


Figure 24. Plot of height by number of shoots in the population 82-158 x 82-160 [(W78-66 x tall cultivar) x (W78-66 x tall cultivar)], where both parental F's were selected for short internodes.

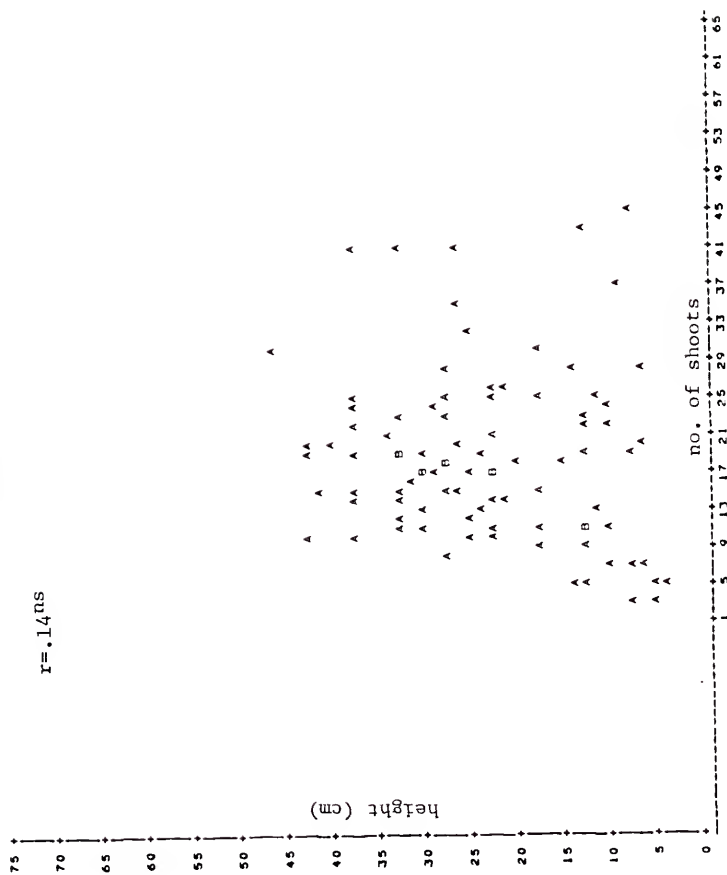


Figure 25. Plot of height by number of shoots in the population 82-160 x 82-161 [(W78-66 x tall cultivar) x (W78-66 x tall cultivar)]₁, where both parental F₁'s were selected for short internodes.

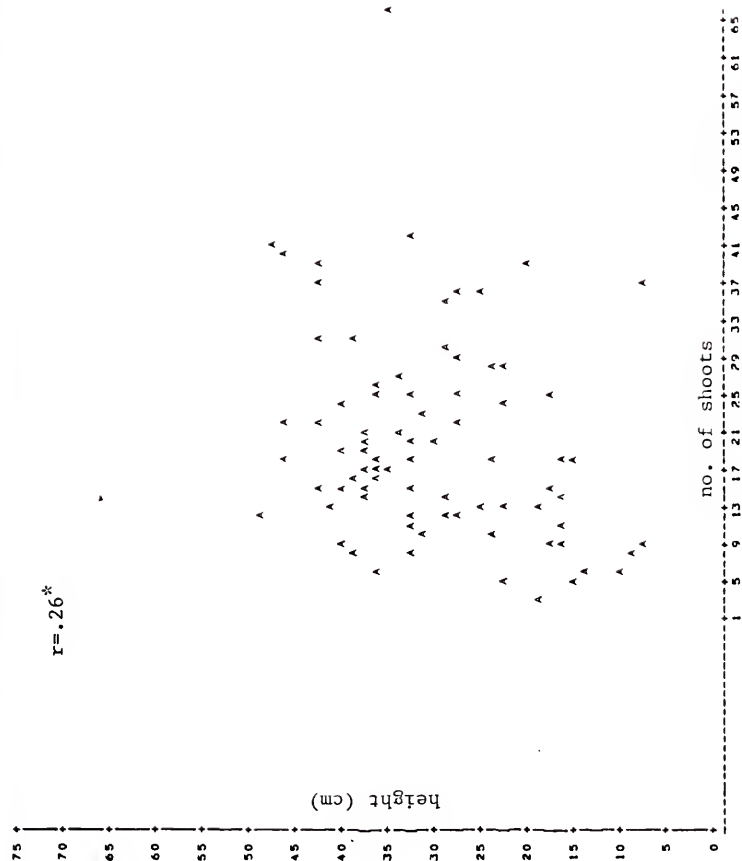


Figure 26. Plot of height by number of shoots in the population 82-161 x 82-156 [(W78-66 x tall cultivar) x (W78-66 x tall cultivar)], where both parental F_1 's were selected for short internodes.

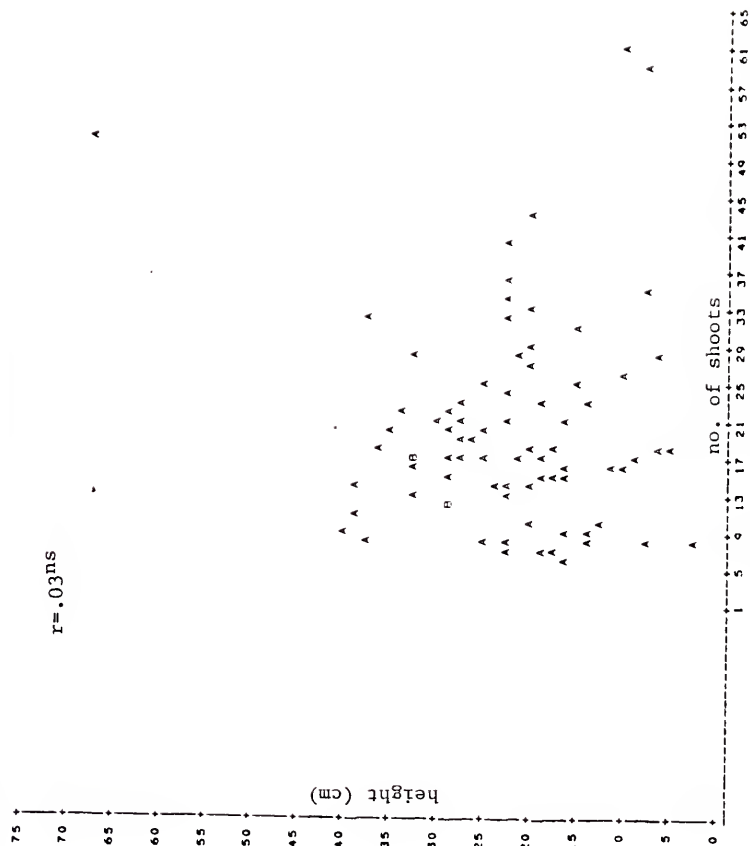


Figure 27. Plot of height by number of shoots in the population 82-195 x W78-66 [(W78-66 x tall cultivar) x W78-66], where the F_1 parent was selected for short internodes.

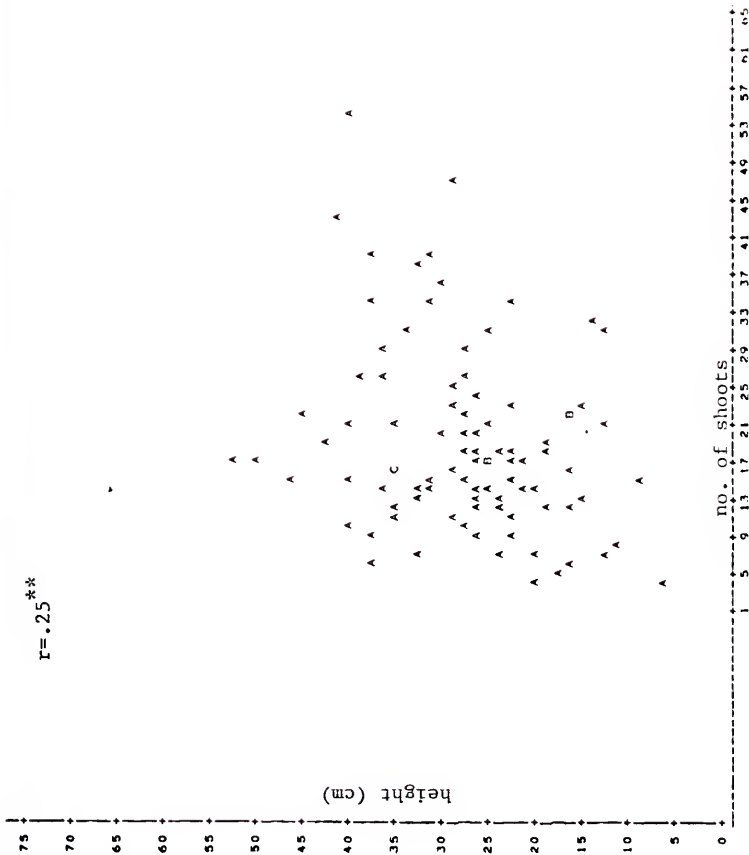


Figure 28. Plot of height by number of shoots in the population 82-196 x W78-66 [(W78-66 x tall cultivar) x W78-66], where the F_1 parent was selected for short internodes.

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BIOGRAPHICAL SKETCH

Edward Garvey was born in La Plata, Maryland, on June 1, 1952. He received his Bachelor of Arts degree from Mississippi State University in July, 1975.

He held management positions at American Garden Cole, Inc., a large wholesale ornamental plant nursery, from December 1974 through July 1979. He was the Propagation Manager at the time of his resignation.

He entered graduate school at Mississippi State University in May of 1979, in the Horticultural Science Department. His major professor was Dr. Thomas Blessington. He studied ornamental horticulture and conducted his research in production of acclimatized interior foliage plants. He received his Masters of Science in horticulture in May of 1981.

He entered the University of Florida in August of 1981. He studied plant breeding and genetics under the direction of Dr. Paul Lyrene. He was awarded the degree of Doctor of Philosophy in December, 1985, in horticultural science (fruit crops).

He is a member of the American Society of Horticultural Science, International Society of Horticultural Science, and the International Plant Propagators Society.

He is married to Angela (Stampley) Garvey and has a daughter, Maria Elisabeth Garvey.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Paul Lyrene

P. M. Lyrene, Chairman
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

W. B. Sherman

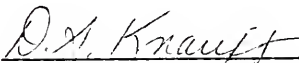
W. B. Sherman
Professor of Horticulture Science

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Gloria A. Moore

G. A. Moore
Assistant Professor of
Horticultural Science

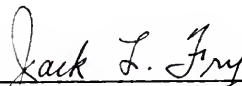
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